

# RIDA® CYCLER

## User manual





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**User manual**

**RIDA®CYCLER**

**ZRCYCLER**

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# User manual

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## General information

Please carefully compare the contents of this delivery with the enclosed delivery note, the package leaflet or the invoice. We recommend that you keep a copy of this document together with the instructions, so that you can quickly access information about the date and scope of delivery in case of future queries, re-orders or service work.

Please be sure to remove all small parts from the packaging material.

We wish to inform you that our devices are adjusted at the factory and can be used immediately after installation.







**Before using the RIDA®CYCLER for the first time, please read the user manual carefully. We recommend that it always be kept with the RIDA®CYCLER.**

## Important information











Before using the RIDA®CYCLER, it is important to read this user manual to familiarize yourself with the instrument. Follow all instructions to ensure proper operation of the RIDA®CYCLER instrument. Do not use any consumables, accessories, or external equipment other than that specified. Safety warnings must be adhered to at all times to avoid risk in personal injury and/or damage to the instrument. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired. The advice given in this manual is intended to supplement, not supersede, the normal safety requirements established in the user's country.

## Explanation of symbols

In these operating instructions, special information is highlighted by the use of symbols:

	Warning! Follow the instructions to avoid risk in personal injury.
	Warning! Follow the instructions to avoid damage to the instrument.
	Warning, electrical hazard!
	Warning, hot surface! The temperature of the rotor may be above 40 °C (104 °F). To avoid injury, do not touch the rotor during a run or 5 minutes following an aborted run
	Biological hazard! There is potential for exposure to infections agents when working with equipment used in molecular biology. To avoid exposure to such hazards, ensure that proper personal protective equipment is worn and that laboratory best practice is adhered to.
	Safety information! Follow the instructions to ensure optimal instrument performance.

## Proper use warnings

	<p>Warning!</p> <p><b>Damaged Lid</b></p> <p>Do not use the RIDA®CYCLER instrument if the lid is broken or if the lid lock is damaged. There is a high risk of personal injury to the user through parts that are moving, electrically live, or are hot.</p>
	<p>Warning, electrical hazard!</p> <p><b>Lethal voltages inside the RIDA®CYCLER.</b></p> <p>When the RIDA®CYCLER is connected to line power, terminals may be live. Opening covers or removing parts is likely to expose live parts.</p>
	<p>Warning, electrical hazard!</p> <p><b>Power Supply Grounding</b></p> <p>Power supply must be connected to an outlet with appropriate grounding means.</p>
	<p>Warning, electrical hazard!</p> <p><b>Main Supply Cord</b></p> <p>Do not replace detachable main supply cord with an inadequately rated cord.</p>
	<p>Warning, electrical hazard!</p> <p><b>Do not clean the chamber with flammable liquids.</b></p> <p>The chamber can reach temperatures above 100 °C (212 °F). Any flammable liquids in the chamber could be a fire risk.</p>
	<p><b>Warning, hot surface!</b></p> <p>In the event of a user aborted run do not open the lid until the instrument has cooled. The rotor within the chamber could be above 40 °C (104 °F). To avoid personal injury, do not touch the rotor for at least 5 min.</p>
	<p>Warning!</p> <p><b>Magnetic Tube Clamp</b></p> <p>Ensure that the magnetic tube clamp is in place before starting a run to ensure the caps and tubes do not come out of the wells during the run.</p>
	<p>Warning!</p> <p><b>Positioning the Instrument.</b></p> <p>Do not position the instrument so that it is difficult to operate the disconnecting device.</p>
	<p>Warning!</p> <p><b>Avoid spilling liquid into the chamber.</b></p> <p>Any solution that spills onto electronic boards could cause a short circuit, damaging the instrument.</p>
	<p>Warning!</p> <p><b>Do not obstruct the side vents.</b></p> <p>Keep the side vents free from obstruction to prevent interference with the cooling of the instrument.</p>



Warning!

**Do not move the RIDA®CYCLER instrument during operation.**

Movement may impair the proper function of the instrument resulting in poor data.



Warning!

**Power Connection**

Avoid removing the power connector from the RIDA®CYCLER before the power indicator light is off on the power adaptor. Failure to do so may result in electrical arcing.



Warning!

**Authorized Service Only**

There are no user serviceable parts inside the RIDA®CYCLER. Service should only be performed by an authorized party.



Warning!

**Power Disconnection**

The power cord set is to be used as a means of power disconnect. Pull the equipment power cord in case of emergency.

## Intended use

The RIDA®CYCLER is intended to be used to perform qPCR or melting, for molecular biology applications with RIDA®GENE, SureFood® and SureFast® assays.

The RIDA®CYCLER is intended for use by laboratory technicians and physicians trained in molecular biology.

RIDA®CYCLER is intended for **Research Use Only**.

The RIDA®CYCLER is a compact rotary based 48-well qPCR instrument that applies magnetic induction to achieve heating and forced airflow for cooling. The instrument comes with four detection channels with excitation and emission spectra that encompass the most common dyes used in qPCR (see also Annex 5: Dye color chart).

With Bluetooth® and USB connectivity to a PC, up to ten RIDA®CYCLER can be run via a single computer increasing sample throughput. Once multiple runs are generated they can be combined into a single analysis of up to ten runs for a total of 480 samples.

## 1 Unpacking and installation

The following items are packaged within the RIDA®CYCLER shipping container:

- RIDA®CYCLER instrument (with tube clamp inside)
- Power adaptor
- Power cable
- 2 m USB cable
- Bluetooth® antenna
- Capping tool
- Mic tubes and caps (960 reactions)
- USB flash drive containing copy of the software and manual
- RIDA®CYCLER Quick Start Guide

### 1.1 Hardware installation

Place the RIDA®CYCLER instrument on a level surface.

Screw in the Bluetooth® antenna at the back of the RIDA®CYCLER; or connect the instrument to a PC using the provided 2 m USB cable.



**Warning!**

**RIDA®CYCLER is not to be used with a USB cable greater than 3 m.**

Plug the power cord into the adaptor and insert the adaptor into the back of the instrument.



**Warning!**

**To avoid electrical arcing, ensure that the power adapter is not plugged into a wall socket before plugging into the instrument.**

Plug the power cord into a wall socket and switch the power on at the socket.

Power the RIDA®CYCLER 'On' using the power switch at the back of the instrument.

An illuminated blue light at the front of the instrument will show the RIDA®CYCLER is powered on and ready for operation.

To switch the instrument 'Off', use the power switch at the back of the instrument.

### 1.2 Software installation

Install the RIDA®CYCLER Software, located on the provided USB Flash drive, onto a PC.

Ensure that the PC meets the following minimum requirements:

- Windows® 7, 32-bit (English version) Operating System
- .NET Framework 4.5 or higher
- Intel i5 processor, 2.4 GHz
- 4 GB of RAM
- 1 GB free hard drive capacity
- Pointer device
- USB Drive
- Adobe® Reader® must be installed to be able to view reports in PDF format.
- Ensure that the PC has Bluetooth® enabled if this is the preferred connection.

In the USB Flash drive menu, double click RIDA®CYCLER.msi software installer.

Follow the instructions that appear in the Setup Wizard.

If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your system administrator.

When the software has been successfully installed, the RIDA®CYCLER software icon will appear on the PC desktop.

Open the RIDA®CYCLER software from the desktop icon.



The software will recognize the instrument via Bluetooth® or USB by displaying the Instrument icon in the tool bar (top right).

Multiple instruments can be recognized by the software and will be displayed.



**Fig. 1:** All connected instruments are now ready to be used.

### 1.3 Updating software

R-Biopharm informs about new software updates via newsletter. Nevertheless, please check the website ([www.r-biopharm.com](http://www.r-biopharm.com)) periodically to see if new software and firmware updates are available or contact your R-Biopharm sales representative.

Download the RIDA®CYCLER software update setup file.

To initiate the installation, double-click on the setup file and follow the prompts.

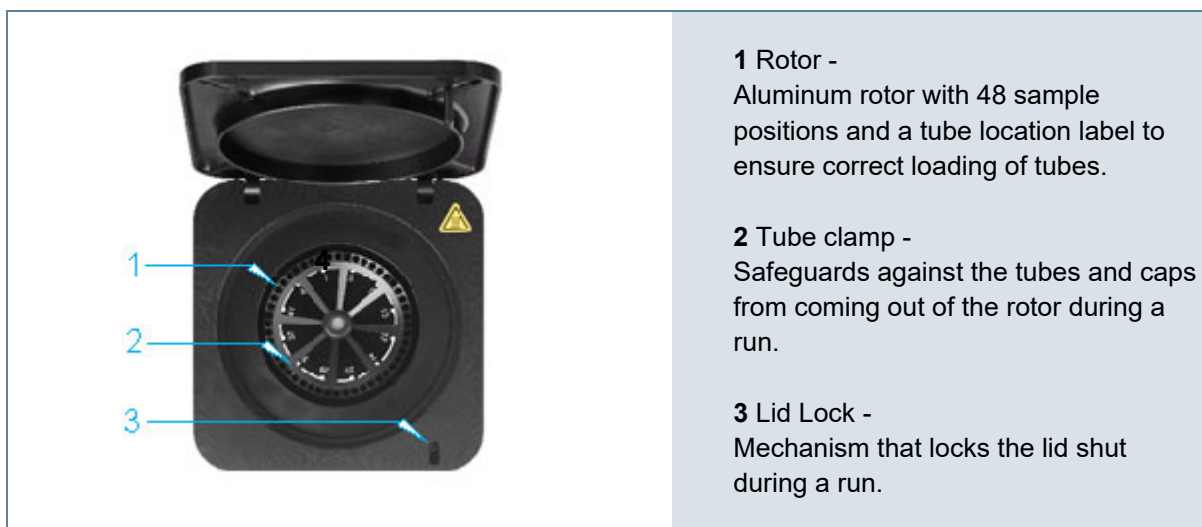
The previous version will be uninstalled automatically.

### 1.4 Upgrading firmware

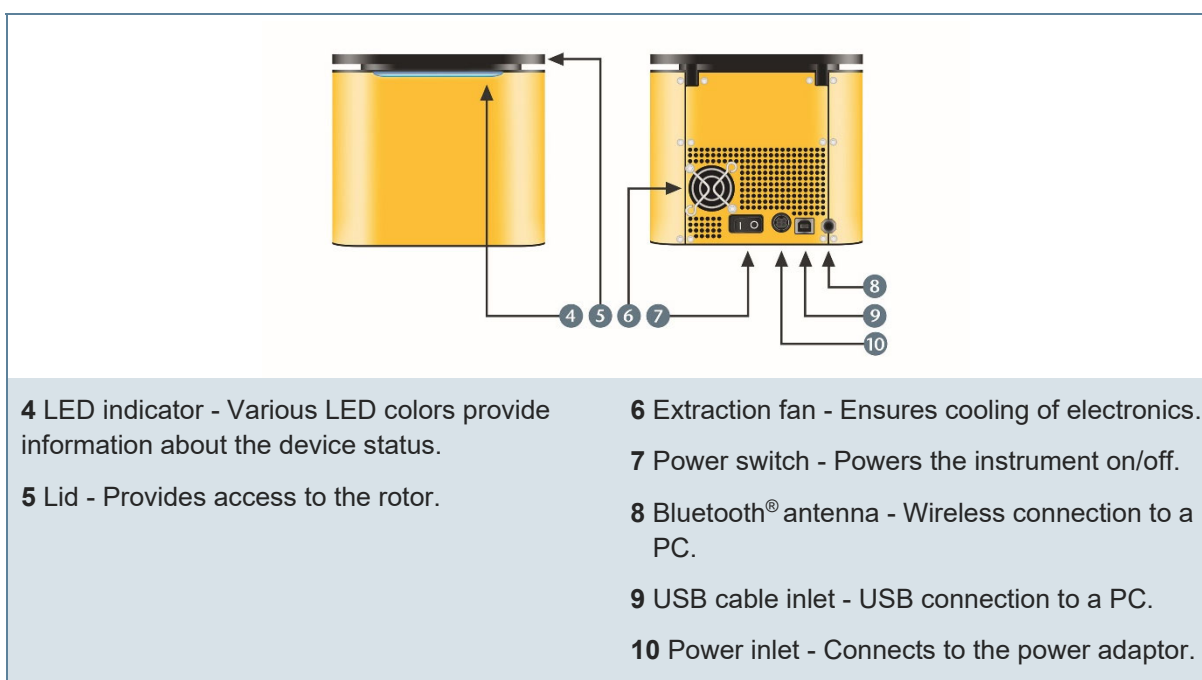
Some new releases of software can require a firmware upgrade. In that case, R-Biopharm will notify the user of the requirement to upgrade the firmware, separately.

To achieve the firmware upgrade, the RIDA®CYCLER must be connected to the PC via USB cable. Firmware upgrades cannot be achieved through Bluetooth®.

## 2 RIDA®CYCLER overview



**Fig. 2:** RIDA®CYCLER top view



**Fig. 3:** RIDA®CYCLER front and back view

### 3 Consumables and accessories

	Power adaptor (Art. No. ZRC-MIC-PA)	External power supply for the instrument. Provided with instrument.
	Tubes and caps (Art. No. ZRC-MIC-TUBES)	Strip of four reaction vessels with a volume range of 5 - 30 µL. Preloaded with silicone oil. Pre-packaged into a rack of 48 tubes, stacked together in a row of 5, and boxed as 4 x 5 stacks. One box provided with instrument.
	Capping tool (Art. No. ZRC-MIC-CT)	Allows for easy insertion of the caps into the tubes. Provided with instrument.
	Loading Block (Art. No. ZRC-MIC-LB)	Aluminum block, allows for convenient loading of the tubes with reagents and sample.
	Mic SBS robotic loading block (Art. No. ZRC-MIC-SBSLB)	SBS dimension loading block to fit most liquid handling systems. Loads 2 x 48 well Mic racks.

**Fig. 4:** Consumables and accessories

### 4 LED indicator colors

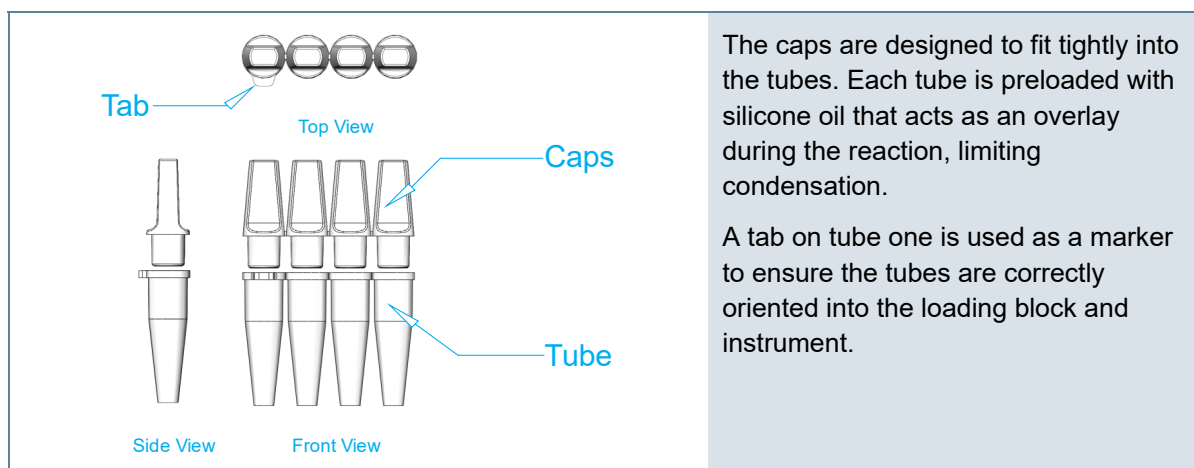
The LED indicator will change color and flash during particular instrument operations.

<b>Blue constant</b>	The RIDA®CYCLER is switched on and <b>Idle</b> .
<b>Blue flashing</b>	The RIDA®CYCLER has been selected to <b>Start</b> a run. This instrument can no longer be selected by another user until the designated run has completed.
<b>Green</b>	The RIDA®CYCLER is <b>Running</b> .
<b>Green flashing</b>	The RIDA®CYCLER has completed the run successfully.
<b>Red flashing</b>	The run has been <b>Aborted</b> , the instrument has had an issue during the run, or the firmware is being upgrade.

## 5 Getting started

### 5.1 Loading tubes

Each tube is part of a strip of four, with the first tube having a small tab to ensure the strip is loaded into the instrument correctly. Each tube is preloaded with silicone oil, which acts as a barrier to prevent evaporation and condensation, thereby improving reaction performance and removing the need for a heated lid. The allowable range of total reaction volume is 5 - 30  $\mu\text{L}$ .



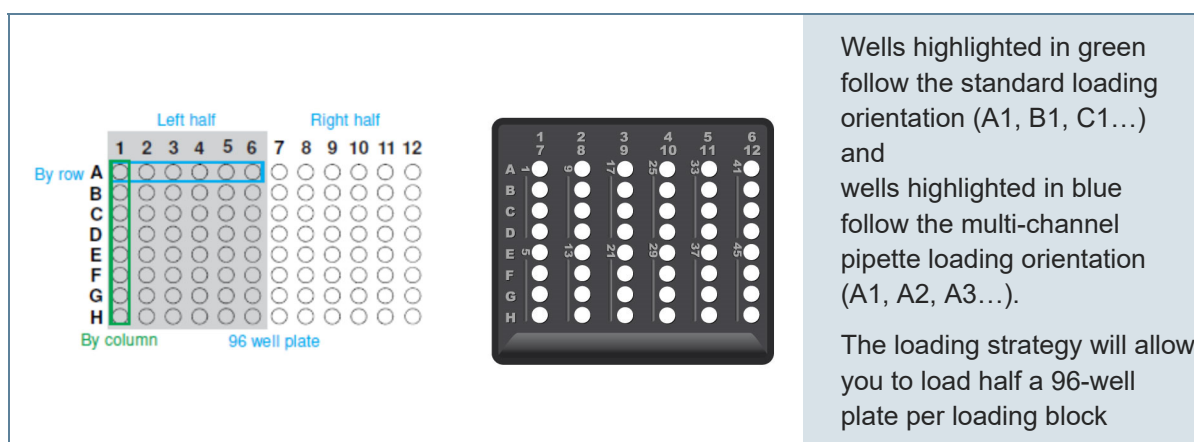
**Fig. 5:** Closing the tubes

**Use the provided loading rack to pipette reagents and samples into each tube.**

The tubes are pre-packed into a loading rack, with the tube tab matching the tab on the loading rack. The loading rack is also compatible with a multichannel pipette (8-channel). For this strategy, the loading orientation switches to across the top of the loading block (A1, A2, A3...) instead of the standard down orientation (A1, B1, C1...). The software has provision to change the display of samples based on the type of layout selected.



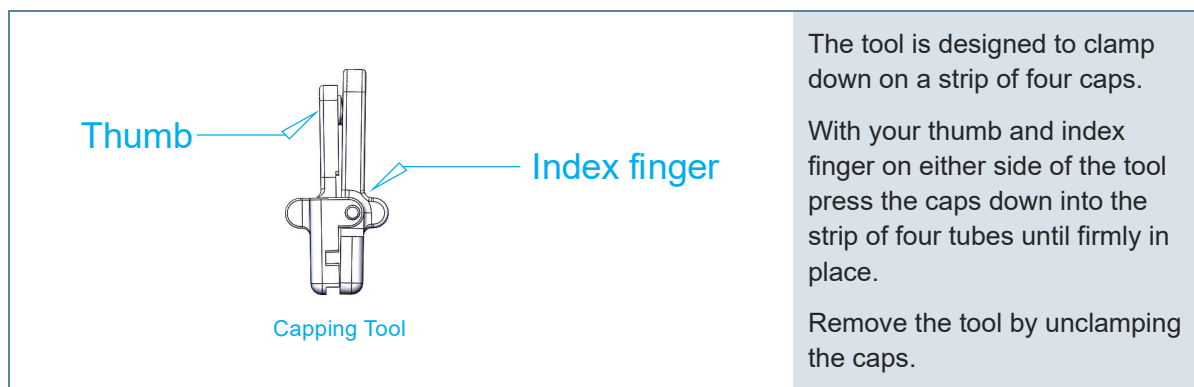
Ensure that only the first six tip positions are utilized and not the full eight.



**Fig. 6:** Loading rack set-up

**Once the tubes are loaded, properly fit the caps to ensure the tubes are sealed.**

Use the provided capping tool to help with fitting caps properly and avoid cross contamination.



**Fig. 7:** Handling of the capping tool

The caps can be removed later to access the post PCR reaction for downstream applications such as gel electrophoresis or DNA sequencing.



**Warning!**

Ensure that post PCR amplicons are handled away from a pre-PCR environment to avoid contamination issues.

**Place the reaction tubes into the rotor keeping the tube tab in line with the marker located on the rotor label.**

**Load water tubes in unused wells.**

No tubes, empty tubes and tubes with different volumes of liquid all have different thermal loads on the metal rotor. Variations in thermal load around the rotor can cause significant thermal gradients both at static temperatures and during ramping, resulting in increased variability in results. For certain analysis methods, even small temperature fluctuations can lead to deviations in the results.

As the oil overlay prevents evaporation, these water tubes can be stored and reused for several runs.



**Safety information!**

To achieve optimum temperature uniformity, it is very important to load tubes, pre-filled with water, into the unused wells of the rotor using the same volume as that of the reaction tubes.

**After loading all the tubes, place the tube clamp at the top of the rotor.**

The magnetic tube clamp will safeguard against any of the tubes or caps coming out of the rotor during a run.

**Once the lid is closed the RIDA®CYCLER is ready to be run.**

Failure to close the lid will prevent the run from starting. This is to prevent injury to the user and/or damage to the instrument.

When the run begins, the lid will be locked into place to prevent it being opened.

## 5.2 Removing tubes

**Once the run has completed and the instrument has cooled down, the lid lock will disengage allowing you to open the lid.**

The LED at the front of the RIDA®CYCLER will flash green.

**Warning, hot surface!**

If the lid lock has disengaged due to a power failure or fault prior to the run stopping, please do not open the lid for at least 5 minutes until the chamber has cooled.

The rotor within the chamber could be above 40 °C (104 °F). To avoid personal injury, do not touch the rotor for at least 5 min.

**Remove the tube clamp and place it to the side.**

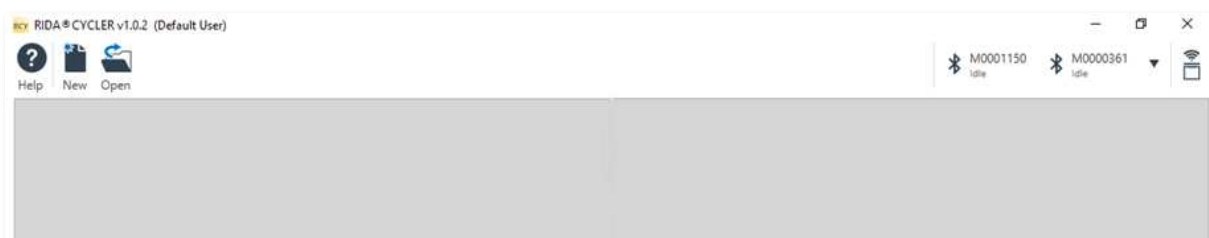
**Pull the tubes out of the wells.**

**Ensure that you put the tube clamp back into the chamber before closing the lid to ensure it is not lost or damaged.**

## 6 Software overview

The software is divided into a number of sections:

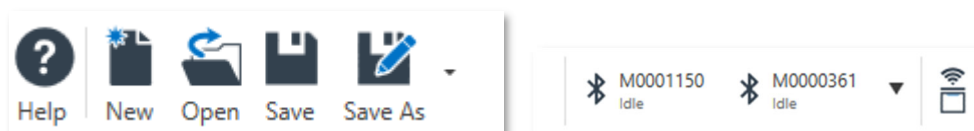
1. Tool bar
2. File tabs
3. Navigator bar
4. File active windows
5. Samples selector



**Fig. 8:** Overview main menu

### 6.1 Tool bar

The top section of the user interface is referred to as the **Tool bar** and consists of the following:



**Fig. 9:** Tool bar overview

<b>Help</b>	Access to <a href="#">RIDA®CYCLER Manual</a> , <a href="#">Create Support Package</a> , <a href="#">Install Template</a> and <a href="#">About RIDA®CYCLER</a>
<b>New</b>	Creating a new <a href="#">Run</a> or start a new <a href="#">Project</a>
<b>Open</b>	Open a saved <a href="#">Run</a> from a file directory
<b>Save</b>	Save an open <a href="#">Run</a>

<b>Save as</b>	Save an open <b>Run</b> under another file name or as a <b>Run Template</b> or <b>Excel Workbook</b>
<b>Instrument</b>	Instruments in communication with the PC are displayed in the tool bar
<b>Instrument Communication</b>	Detect available instruments via USB or Bluetooth

### 6.1.1 Help icon

The **Help icon** is used to access the following options:

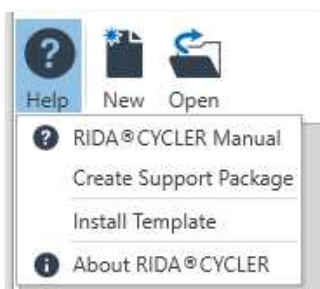


Fig. 10: **Help icon** overview

<b>RIDA®CYCLER Manual</b>	An electronic version of the user manual is stored within the software.
<b>Create Support Package</b>	Create a support package after experiencing any fault with the software or hardware. The support package contains a compressed log file of the run. Select a folder to save the support package to Email the zipped support package file to <a href="mailto:pcr@r-biopharm.de">pcr@r-biopharm.de</a> .
<b>Install Template</b>	Load templates provided by R-Biopharm into the RIDA®CYCLER software via "Install Template". The templates provided by R-Biopharm already contain all required thermal profile settings.
<b>About RIDA®CYCLER</b>	Information about the version of RIDA®CYCLER software.



**Safety information!**

Before starting a run, the necessary templates and assays need to be installed.

### 6.1.2 Open run files

There are different file types:

**.rcyclerrun (Run File):** contains the assays used, run profile, sample annotation, raw data and analyzed data (toothed wheel).

**.rcyclertemplate (Run Template):** contains a complete pre-run, including sample annotation. A template can be used for repetitive runs using the same sample layout each time. Use **New** to open a run template.

**.rcyclerproj (Projects):** analysis of a combination of compatible runs.

### 6.1.3 Instrument icon

Instruments in communication with the PC will be displayed in the tool bar as an **Instrument icon**.

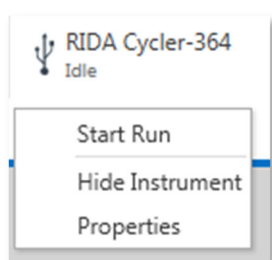
A Bluetooth® or USB symbol will indicate the type of communication achieved.

The serial number or name of the instrument is displayed next to the communication symbol.

The status of the RIDA®CYCLER is also displayed beneath the name:

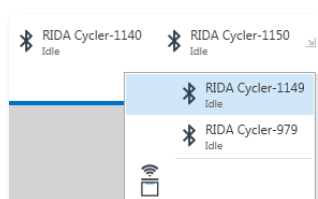
<b>Idle</b>	RIDA®CYCLER can be used to start a run.
<b>Setup</b>	Someone has transferred run information to the instrument from another connected PC but has not yet started the run. This instrument cannot be used until the run has completed or aborted.
<b>Running</b>	RIDA®CYCLER is running and cannot be used until the run is completed.
<b>Offline</b>	RIDA®CYCLER has lost communication with the PC.
<b>Reconnecting</b>	RIDA®CYCLER is regaining communication with the PC (during a run).

Click on the instrument icon to display the following options:



**Fig. 11:** Instrument icon overview

<b>Start Run</b>	Runs begin by selecting the <b>Start Run</b> option. The <b>Start Run</b> option will only appear when a <b>New Run</b> has been initiated.
<b>Hide Instruments</b>	Select <b>Hide Instrument</b> if you do not wish to display a particular instrument in the software. Use this option if you want to avoid cluttering your PC with other instruments you are not using but are in communication with.
<b>Unhide</b>	To unhide an instrument, select the <b>down triangle</b> to display a list of hidden instruments; then select the instrument you wish to unhide.



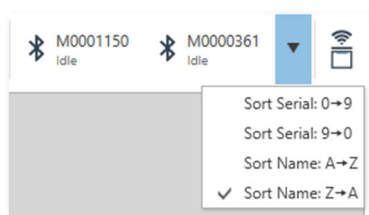
**Fig. 12:** Hide instrument option

<b>Properties</b>	Change the name of the instrument; and there is also information regarding the serial number and firmware version.
<b>Update Firmware</b>	Updating of the instrument firmware. The option is only available after specific RIDA®CYCLER software upgrades.

Some options are only displayed when required:

<b>Reconnect Run</b>	Lets the PC reconnect to a run following a dropout when using Bluetooth®.
<b>Recover Run</b>	Obtains the run from the instrument if connection has been interrupted and the run has been completed. The instrument will store the data until such time.
<b>Reconstruct Run</b>	Allows the user to view the run on another PC connected to the instrument that did not start the run.
<b>Temperature Verification</b>	Allows you to determine if the instrument is within specification using the Temperature Verification System (TVS). See Appendix A on how to operate the TVS. This option will only appear if the TVS is plugged in via USB.

In the toolbar, instruments recognized by the system can be sorted by name or serial number. To do this, click on the arrow to the right of the listed devices and select your desired sorting.



**Fig. 13:** Instrument sorting function

#### 6.1.4 Instrument communication icon

The instrument communication icon is used to search for nearby instruments via both Bluetooth® and/or USB.

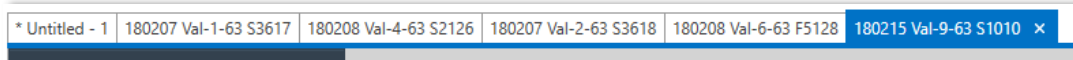
On software start up the icon will flash automatically indicating the software is searching for instruments.

The maximum range for the Bluetooth® antenna is approximately 7 m with no obstruction from solid walls.



**Fig. 14:** Instrument communication icon

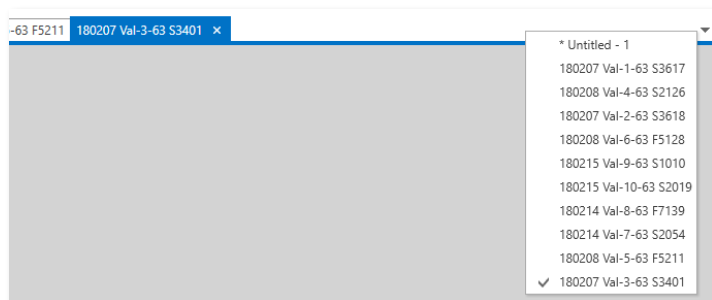
## 6.2 File tab



**Fig. 15:** File tabs

Every open file will be displayed with its name on a tab. Multiple files can be open at the one time. The file being displayed in the main window will be highlighted in blue. Files that need to be saved will have an asterisk just before the file name. If a file is linked to a run in progress, selecting the instrument running it will open the associated tab.

Use the down arrow to view files that might be out of view if too many tabs are open at the one time.



**Fig. 16:** File tab overview

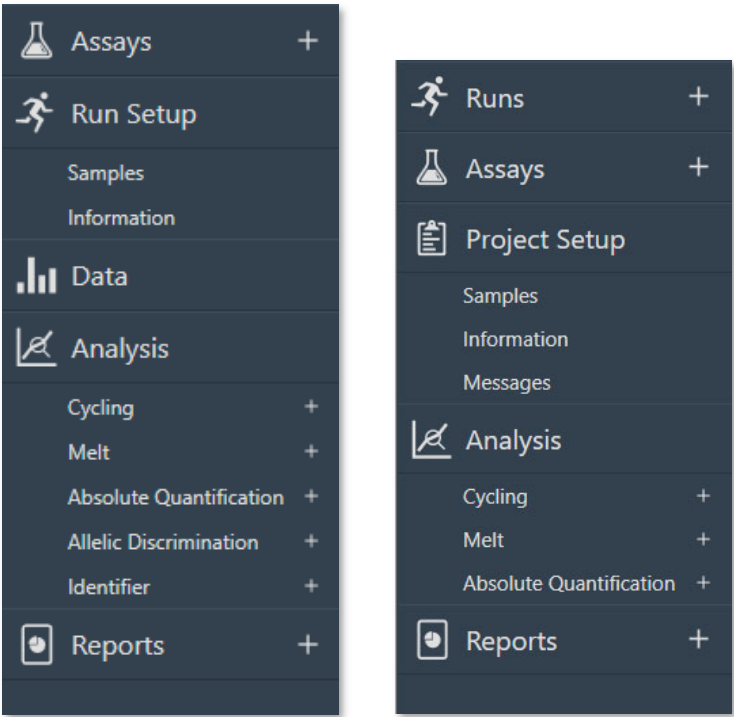
## 6.3 Navigation bar

To the left-hand side of the main user interface is the **Navigator bar**. The **Navigator bar** allows you to view the different sections for an **Assay** or **Run**.

Some sections contain subsections that can be viewed by expanding the navigator tree.

Sections that are open in the main window will be highlighted in blue. Important sections specific to the file are emphasized in bold.

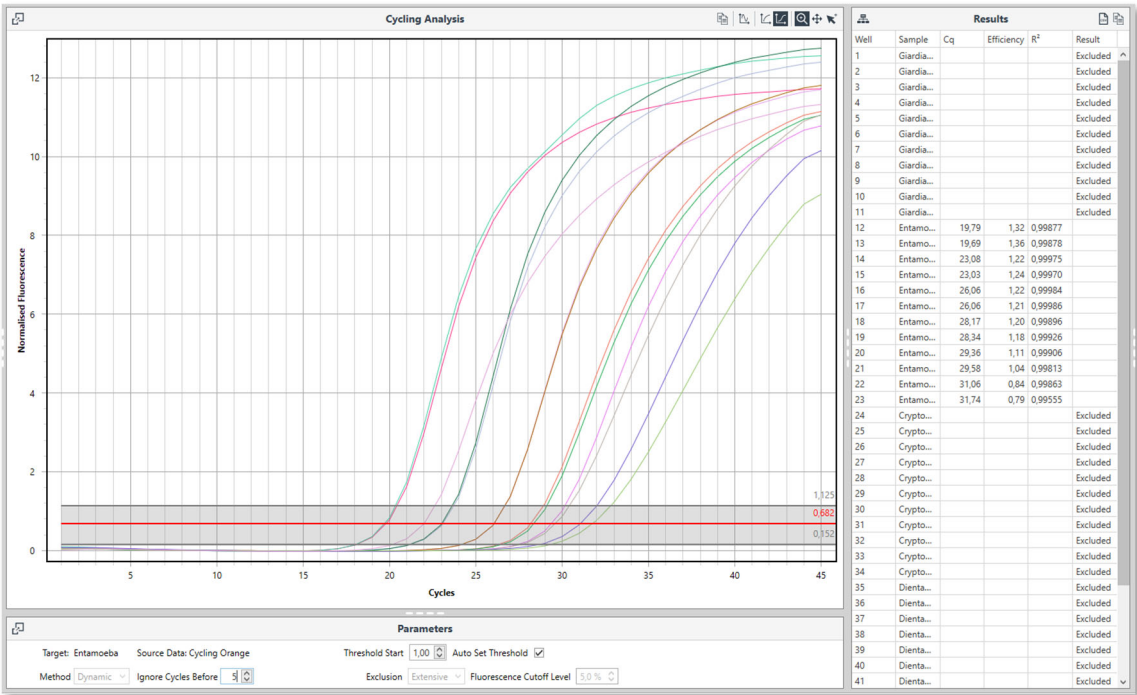
Remove Assays and Analyses by using the **delete icon**.



**Fig. 17:** Navigator bar with assay selection, run setup, data, analysis and report section (*left*); navigator bar for projects (*right*)

#### 6.4 File active windows

In the central area of the user interface are segmented windows that are active for a specific section of the navigator bar.

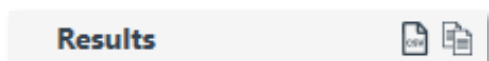


**Fig. 18:** Cycling analysis window showing the results of an experiment

<b>Raw Data</b>	The data is updated in real-time during the run and is available after the run has completed. The graphs are scaled automatically during a run and can be scaled manually by expanding or contracting each axis.
<b>Analysis graph</b>	Analyzed data displayed in a specific graph type depending on the analysis chosen. The graphs can be manually scaled by moving each axis.
<b>Analysis Parameters</b>	can be changed to optimize for the target being analyzed
<b>Results table</b>	Numerical representation of the analyzed data. The table can be setup to display the samples as replicates, with mean and standard deviation, or as individual wells.

#### 6.4.1 CSV Export or copy to clip board

Each **Result table** and **Samples editor** will have two options for copying the data for easy export to third party software:



**Fig. 19:** Result table

<b>CSV</b>	Save the results as a CSV file.
<b>Copy to Clip board</b>	Copy the results to the clipboard, then paste into another third-party software such as Microsoft® Word®.

#### 6.4.2 Export raw data

The raw data for Cycling can be exported by selecting the Save the data as a CSV file icon.



**Fig. 20:** Symbol for saving data as CSV file

#### 6.4.3 Export chart as bitmap

Copy the graphs to a bitmap format for pasting into any third-party document processing software (e.g. Microsoft® Word®).

Scale the image to the size required.

To ensure you retain as much detail in the image as possible adjust the image to the largest view possible.

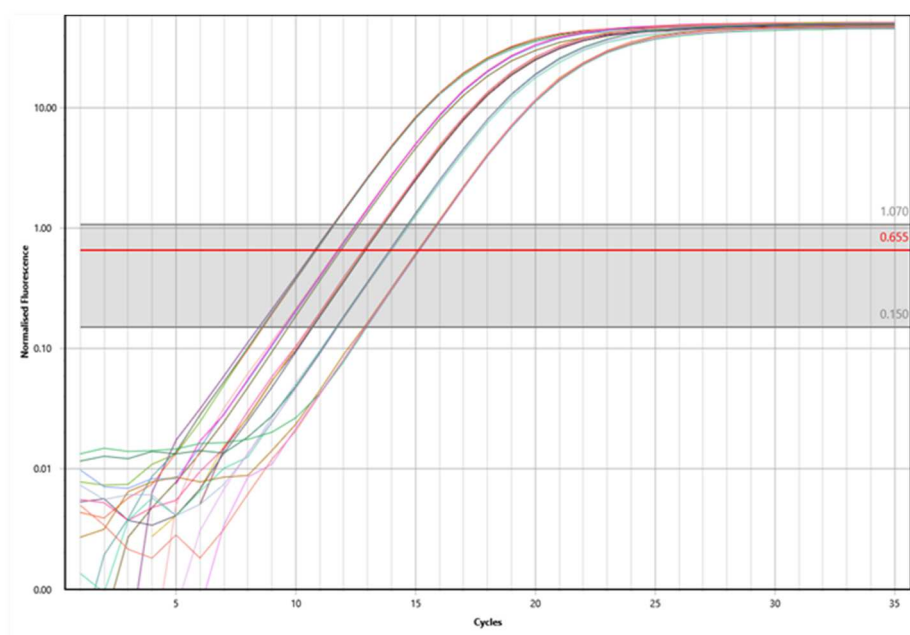
Select the Copy chart to the clipboard icon.



**Fig. 21:** Symbol for copying charts to the clipboard

Paste the image into the third-party document processing software as a bitmap.

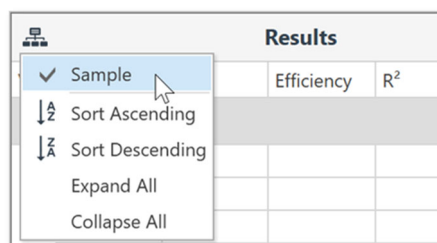
The image should be of sufficient quality for publication purposes.



**Fig. 22:** Result image as bitmap

#### 6.4.4 Result table organization

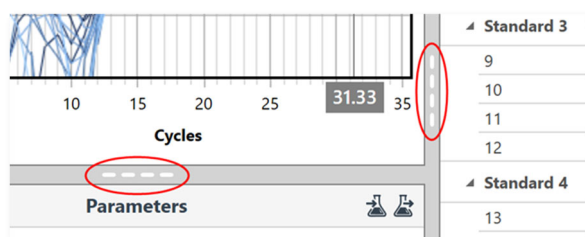
Each column can be sorted in ascending or descending order by clicking on the column or by right clicking on the column.



**Fig. 23:** Sample sorting options

### 6.4.5 Sizing Bars

The width or height of these windows, relative to each other, can be adjusted using the sizing bars.



**Fig. 24:** Available sizing bars are indicated as white dashed lines, here marked in red.

### 6.4.6 Graph display functions

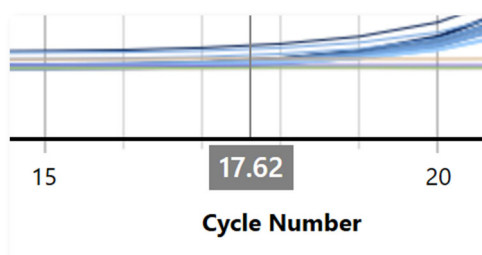
There are three graph display functions available:



**Fig. 25:** Graph display functions

<b>Zoom</b>	Magnify a chosen area to view more detail within the graph. To zoom back out, double click anywhere on the graph.
<b>Pan</b>	Combining the Zoom function with <b>Pan</b> allows you to move the display around a magnified field of view allowing you to locate and focus on specific areas of the graph
<b>Select samples</b>	Only the <b>Select samples</b> will be displayed on the graph

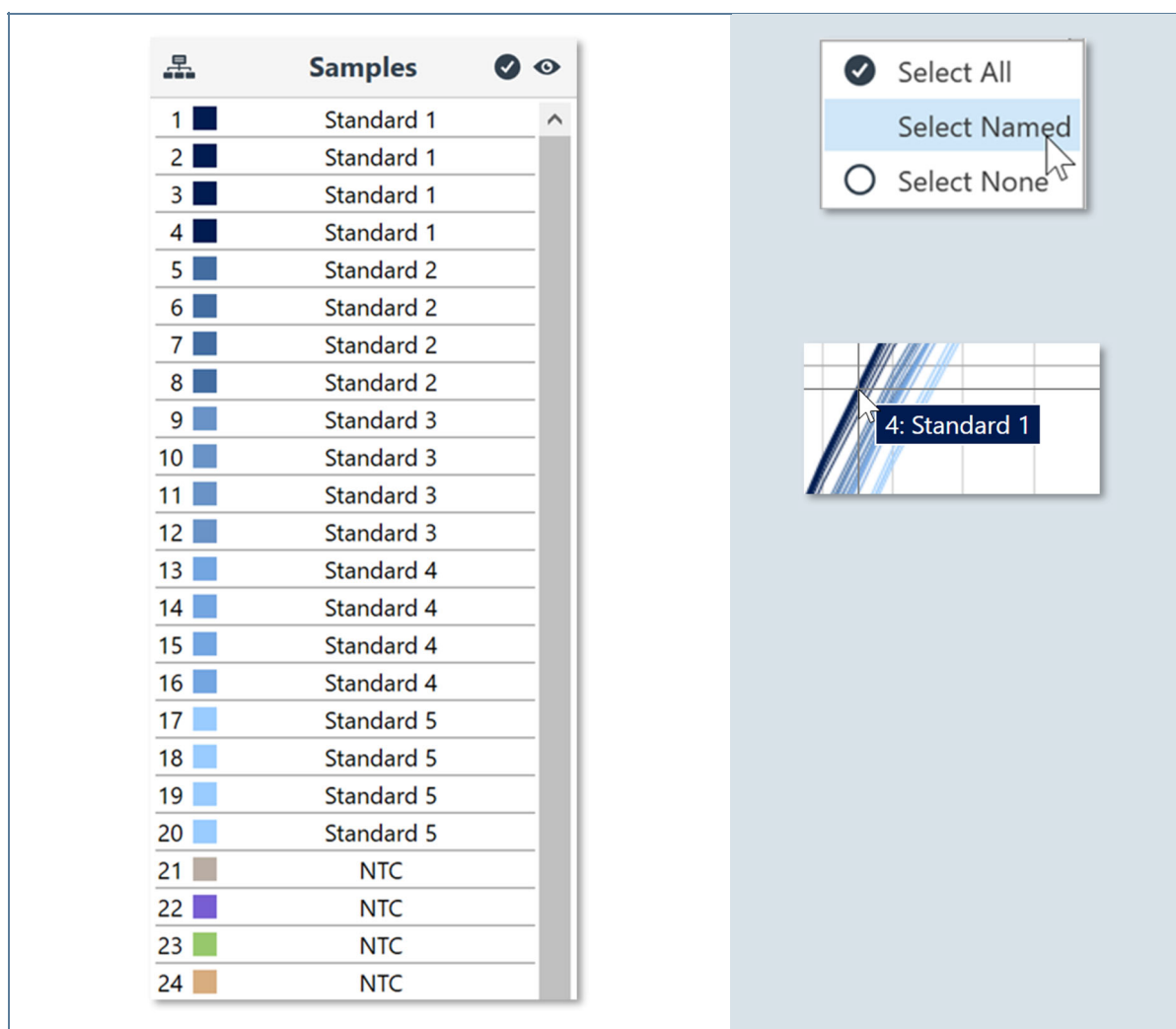
**Cross hairs:** displays the coordinates of any point inside the Cycling analysis window. Values are given in a grey text box along each axis.



**Fig. 26:** Cross hairs

## 7 Samples selector

Deselect and select specific samples using the **Samples selector** located to the right-hand side of the user interface. Select/deselect individual samples by clicking on the sample bar. Alternatively, select just the named samples using the **Select Name**, or select all samples by using the **Select All**, or deselect all of the samples by using the **Select None** options.

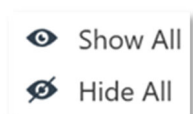


**Fig. 27:** Sample selector bar

Hovering the mouse cursor over a sample in a graph will highlight the sample by drawing the line thicker and displaying the name of the sample in a small text box along with the well-number. The sample bar in the **Samples selector** is also highlighted in black. Similarly, hovering over a sample in the Samples selector will highlight the sample in the graph.

Use the sample selector to remove or reinstate samples from analysis. The removal or addition of samples from analysis may change the position of the W-o-L (Window of Linearity) and therefore the automatic cycle threshold. Poorly amplified samples can affect the performance of the LinRegPCR algorithm, preventing it from determining a W-o-L. Removal of such samples from the analysis may allow for the determination of the W-o-L.

To only hide a sample from view within a graph, and not remove it from the analysis, use the **View icon** located on the sample selector. There is also an option to **Show All** or **Hide All**.



**Fig. 28:** Show / hide function

7.1 Samples selector grouping

Select the Samples Selector Grouping icon to view the sample selector based on Assay, Sample Name or None (well order).

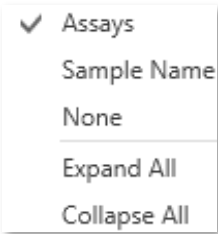


Fig. 29: Sample grouping

This option makes it easier to view or hide samples based on grouped types. You also have the option to Collapse or Expand the groupings to minimize cluttering on the sample selector.

















Samples		<input checked="" type="checkbox"/>	
▶	Control	<input checked="" type="checkbox"/>	^
▶	(None)	<input type="checkbox"/>	
◀	Treatment Regional	<input checked="" type="checkbox"/>	
6	 Sample 6		
7	 Sample 7		
8	 Sample 8		
9	 Sample 9		
10	 Sample 10		
22	 Sample 6		
23	 Sample 7		
24	 Sample 8		
25	 Sample 9		
26	 Sample 10		
38	 Sample 6		
39	 Sample 7		
40	 Sample 8		
41	 Sample 9		
42	 Sample 10		
▶	Treatment Urban	<input checked="" type="checkbox"/>	

Fig. 30: Sample overview

## 8 Creating a new run

Select **New** from the tool bar menu and then **template for the new run** from the drop-down list.

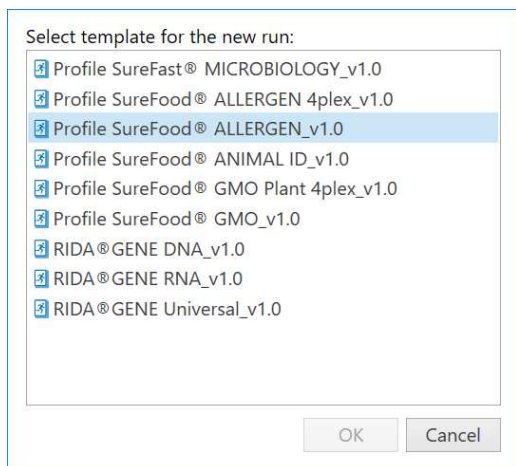


Fig. 31: Template window

### 8.1 Adding assays

Select the **Assays** required for the run by selecting the **Add** button.

You can select from any library of assays displayed next to the navigator bar. By default, the RIDA®CYCLER Library will be displayed.

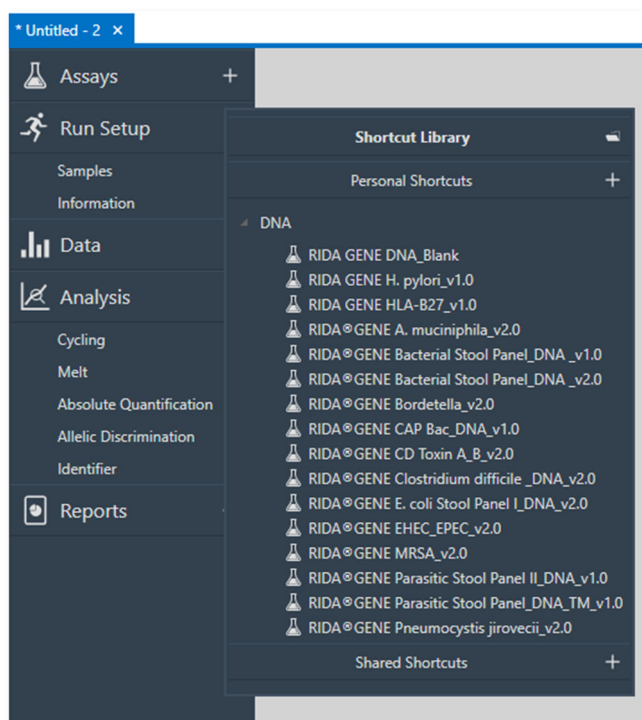
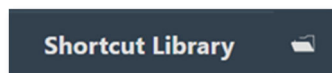


Fig. 32: Navigator bar with assay library

Select an assay from any directory using the file icon.

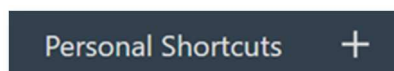
Use the file explorer to locate the assay you require from any location including network drives or external hard drives such as a USB Flash drive.



**Fig. 33:** Shortcut library

**You can create a new assay library Shortcut.**

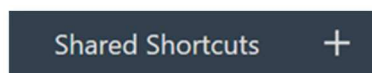
Select the **+** button next to **Personal Shortcuts** then browse and select the location of the new directory you wish to use as a personal assay library.



**Fig. 34:** Creating an assay shortcut

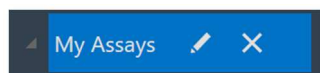
**Create a Shared assay library shortcut.**

If you have multiple users sharing a PC or network drive you have the option to create a shared location for all of your assays.



**Fig. 35:** Shared shortcuts

**You can Edit or Delete assay libraries from the list.**



**Fig. 36:** Personal assay library

The **Assay Profile**, **Analysis Settings** and **Information** can be viewed once the assay is selected. Remove assays by using the delete button next to the assay name.

## 8.2 Assay profile compatibility

If an assay is selected that has a thermal **Profile** not compatible to the template profile, the software will bring up the following warning message:

“The selected assay is not compatible with the run. The assay’s profile and current run’s profile do not match and cannot be automatically adjusted. Please indicate how you wish to proceed:”

Select from the following options:

<b>Modify Run</b>	The run’s profile will be modified to make it compatible with the selected assay. This may cause existing assays to become incompatible.
<b>Modify Assay</b>	The assay’s profile will be adjusted to match the run.
<b>Add Without Modification</b>	The selected assay will be added without modification. The assay will remain incompatible until it or the run’s profile is modified.
<b>Cancel</b>	Do not add the assay to the run.

Non-compatible assays are reported with a caution symbol.

Specific reasons for the non-compatibility are reported below the symbol. You can also enter the **Profile** for the incompatible assay and select **more...** to view all of the reasons. In the area, you have the choice to:

Modify the run to match this profile: The run's profile will be modified to make it compatible with the selected assay. This may cause existing assays to become incompatible.

Modify this assay to match the run profile: The assay's profile will be adjusted to match the run.



**Fig. 37:** Warning if assay and run profile are incompatible from the template.

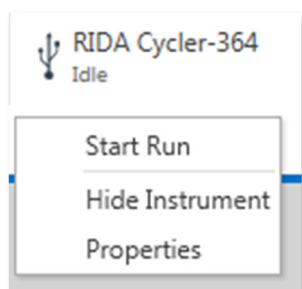


Test any modified profiles on a small subset of samples to confirm these changes are valid, before running experiments with a large number of samples to avoid major loss of valuable samples and reagents. The consequences might be suboptimal performance of the qPCR leading to poor results.

## 9 Starting the run

Select the **Instrument** you wish to use for the run in the tool bar.

Only **Idle** instruments can be selected to start a run.



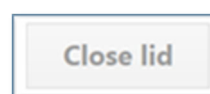
**Fig. 38:** Available instruments are displayed in the tool bar.

Once the instrument is chosen, begin the run by selecting the **Start Run** option from the drop-down list.

A confirmation dialogue box will appear.

Ensure that the tube clamp is in place to prevent the tubes and caps from coming off during a run.

Ensure that the lid is closed prior to starting the run. A lid sensor will detect if the lid is open and will prevent the instrument from starting, while a warning will notify you of the fact



Double check that the volume displayed is correct.

At this point the instrument LED indicator will be flashing blue indicating to other users the RIDA®CYCLER has been 'booked' to start a run. No other user can start a run on the instrument until it has completed this run. On another user's PC, the instrument will be displayed as Busy, when they go to select it.

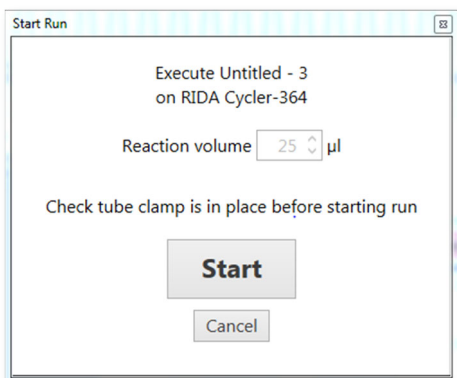


Fig. 39: Start Run window

To execute the run, click the **Start** button in the **Start Run** dialogue box.

The instrument will automatically lock the lid, centrifuge the samples down, and then the run profile will begin. The LED indicator will turn green to notify a user the RIDA®CYCLER is running.

## 9.1 Samples editor

The **Samples editor** is displayed in a table format and allows you to annotate your samples. Samples can be annotated before, during or after a run. Failure to properly annotate samples can affect analysis. A default table is provided but the position of the columns can be changed by dragging a particular column header left or right.

### 9.1.1 Well layout

The well layout can accommodate loading from a 96 well plate using a multichannel pipette, where the orientation switches from columns to rows. To ensure the software displays this change, select from the options below.

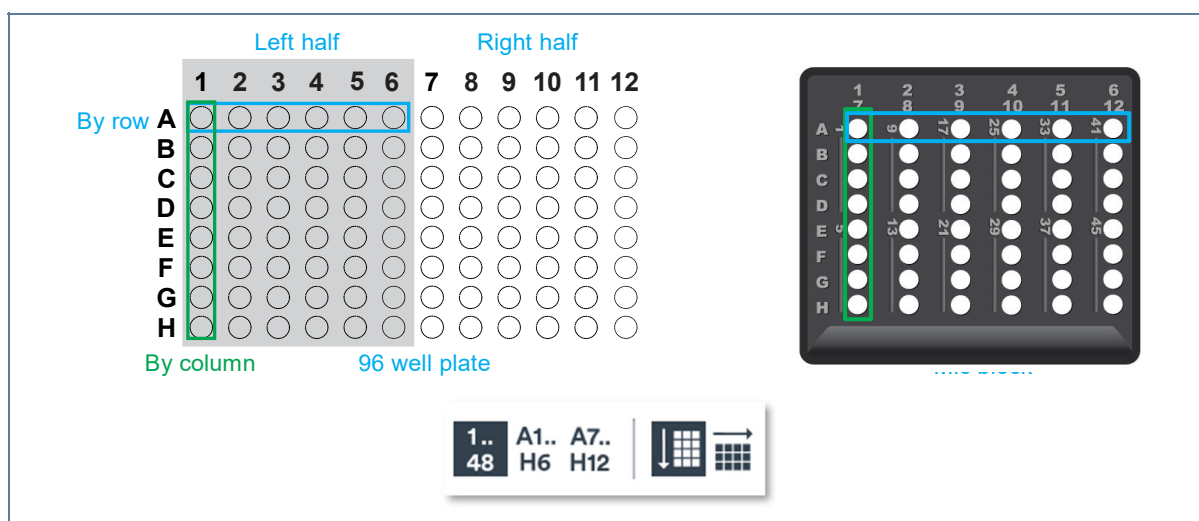


Fig. 40: Well layout

Display wells as either numerical (1, 2, 3...) or alpha numeric (A1, B1, C1).

If loading from a 96 well plate, select if the samples are displayed from the left half of the 96 well plate (A1 – H6) or the right half (A7 – H12).

If loading from a 96 well plate using a multi-channel pipette, change the orientation of the display from '...by column' to '...by rows'.

### 9.1.2 Filling cells

Cells can be filled individually or in groups.

Using the **Enter** key on your keyboard will move to the next cell down.

Using the **Tab** key will move to the next column.

Use the delete key to clear a cell.

Copy and then paste names and concentrations from other software programs (e.g. Microsoft® Excel®).

### 9.1.3 Colors

Select the **Color** you want for each sample (optional).

Chose any color from the color pallet or generate your own colors using the color chart.

To create a gradient, select the first color and highlight all the way down to the last color required, and then click the **Auto fill** icon.



**Fig. 41:** Auto fill icon

### 9.1.4 Name

Enter the **Name** of each sample.

Samples with the same characters and assay will be treated as replicates and will be reported with a mean ( $\bar{x}$ ) and standard deviation ( $x\sigma_{(n-1)}$ ) in most analyses.



Samples with the same characters but different assays will be linked based on the type of analysis chosen.

You can highlight multiple cells within a column and enter the same characters to annotate replicates. Alternatively, enter the name in one cell, highlight that cell and other cells that will be part of the replicates (use Ctrl + Click to highlight non-adjacent cells), and then select the **Fill down** icon to give all the selected cells the same name.








**Fig. 42:** Fill down icon

















Use the **Auto fill** icon to annotate sequential characters (e.g. sample 1, sample 2, sample 3...). To allow for replicates follow the following process:

Enter the first set of characters for the first name (Sample 1).		Colour	Name
	1		Sample 1
	2		


  

Leave the same number of rows blank as the number of replicates required below the first name. Enter the second name of the sequence (Sample 2).		Colour	Name
	1		Sample 1
	2		
	3		
	4		Sample 2
5			

















  

Now highlight all the cells required to complete the filling of the names and replicates.		Colour	Name
	1		Sample 1
	2		
	3		
	4		Sample 2
	5		
	6		
	7		
	8		
	9		
	10		
	11		
	12		
	13		
	14		
	15		
16			

Click on the Auto fill icon.	
------------------------------	---

The names will be sequential based on the first two inputs and the replicates for each will be automatically filled in too.		Colour	Name
	1		Sample 1
	2		Sample 1
	3		Sample 1
	4		Sample 2
	5		Sample 2
	6		Sample 2
	7		Sample 3
	8		Sample 3
	9		Sample 3
	10		Sample 4
	11		Sample 4
	12		Sample 4
	13		Sample 5
	14		Sample 5
	15		Sample 5
16			

**Fig. 43:** Sample name options

### 9.1.5 Sample type

#### Select the sample **Type**.

There are seven options to choose from. The type chosen will determine the way in which the sample is utilized during analysis.

To change multiple cells at once, highlight the cells, use the F2 key on your keyboard, and then select from the following options:

<b>Unknown</b>	Default for any sample that is under investigation.
<b>Standard</b>	A sample of known quantity, used to generate a standard curve from which an unknown sample quantity can be calculated, or used to determine amplification efficiency.
<b>Positive Control</b>	The sample is known to contain the target of interest. A positive control is used to confirm that the assay is working and helps prevent false negatives.
<b>Negative Control</b>	The sample is known not to contain the target of interest. A negative control is used to monitor for contamination of the assay and is helpful in preventing false positives.
<b>Extraction Control</b>	The extraction control verifies that the DNA extraction was carried out without any contamination.
<b>NTC (No Template Control)</b>	A sample that contains no target genes. NTC's are used to detect amplicon contamination. The NTC may contain the negative control of an internal amplification control template to ensure that the PCR is working.



The concentrations will be filled based on the first two inputs and the replicates for each will be automatically filled in too.

Concentration
Units <input type="text" value="Copies/μL"/>
20000
20000
20000
10000
10000
10000
5000
5000
5000
2500
2500
2500
1250
1250
1250

**Fig. 44:** Sample concentration options

### 9.1.7 Multiplex Standards

You can enter standard concentrations for each channel individually.

Select the **Toggle between single and multiplex standards** icon to use the option of entering multiplexed standards. You must ensure that the assay has been setup as a multiplex.

Switching between single and multiplex is possible; however, only one type can be viewed and applied during analysis.



**Fig. 45:** Icon for toggling between single and multiplex standards

Green Channel Standard...	Yellow Channel Standard...	Orange Channel Standar...	Red Channel Standards...
<input type="text" value="%"/>	<input type="text" value="%"/>	<input type="text" value="%"/>	<input type="text" value="%"/>
9	35	1	55
1	55	9	35
25	25	25	25
55	9	35	1

**Fig. 46:** Standard concentrations by using multiplex standards in all channels

### 9.1.8 Linking an assay to a sample

An assay must be linked to a sample to allow the software to recognize and properly analyze the sample. Failure to allocate an assay to a sample will result in the sample not being analyzed.

**Link one Assay to a sample.**

Select the required samples by highlighting the cells in the **Assays** column. Use Ctrl + Click to highlight non-adjacent samples.

Select the required assay from the **Available Assays** window then drag and drop the assay into the highlighted cells. Alternatively, click on the selected cells in the **Assays** column and use the drop-down list to select the required assays(s) by ticking the box next to the assay name, or **Select All**, and then click the **OK** button.

To remove an assay, form the Assays column, use the delete key of your keyboard or click on the cell to bring up the drop-down list. Then simply un-check the assay from the list provided to remove it, or **Select All** to remove every assay.

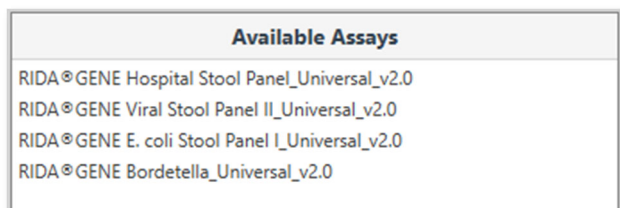


Fig. 47: **Available Assays** window

### 9.1.9 Optional columns

Additional columns can be added to the **Samples editor** using the **Select visible sample data columns** icon. The following columns can be added or removed from the table:

**Standard Concentration:** concentrations are used for Standard curve analysis.

**RIN:** provide a RNA integrity number for each input RNA.

**Comment:** provide additional information about the samples.

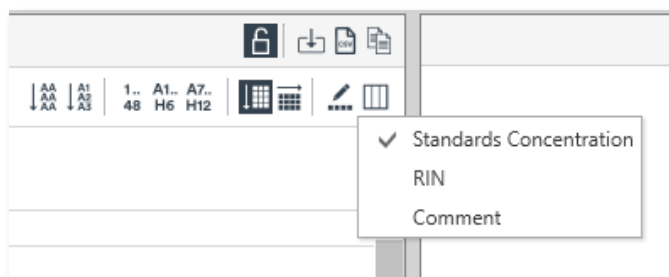


Fig. 48: **Select visible sample data columns**

### 9.1.10 Import samples

For a convenient sample annotation import sample information from a number of different file types.

	A	B	C	D
1	Well	Sample ID	Concentration	
2		1 Sample 1	82	
3		2 Sample 2	158	
4		3 Sample 3	569	
5		4 Sample 4	924	
6		5 Sample 5	717	
7		6 Sample 6	731	
8		7 Sample 7	199	
9		8 Sample 8	110	
10		9 Sample 9	184	
11		10 Sample 10	812	
12		11 Sample 11	113	
13		12 Sample 12	246	
14		13 Sample 13	711	

**Fig. 49:** Example for sample information

Select the **Import Samples icon** to import sample information from another source.

You can import from any comma delimited, tab delimited or space delimited files. Browse and select the file to import.



**Fig. 50:** Import Samples icon

Select the fields to import and into which column of the Sample Editor.

Once the run file is selected a table will display all the fields in the file. You have the option to select the type of delimitation (e.g. comma) and how many rows should be ignored before capturing the data. Next, choose the fields to import by linking the column from the files to one of the columns in the samples editor using the drop down menu.


**You have the option to save the import style as a template.**

This will allow you to complete the import faster without having to re-do the matching when conducting repetitive runs.

#### 9.1.11 Sample editor warnings

Various warnings will be displayed, if annotations have not been completed correctly. Some examples include:

- If standards have been selected as **Type** but no values have been entered into the **Standards Concentration** field.
- Assays have not been linked to an edited Sample row.

 The standard in well 1 does not have a concentration defined.  
Some named samples do not have an assay assigned to them.

**Fig. 51:** Possible warning message

### 9.1.12 Lock down sample editor

All of the fields can be locked down to prevent accidental changes. Once locked, none of the fields are annotatable until unlocked again.



**Fig. 52:** Icon for locking down the sample editor

### 9.1.13 Information

**Enter the name of the Operator (Optional).**

**Enter any Notes about the run (Optional).**

Provide enough detail to help you understand the run at a later time.

Details may include the experimental purpose of the run or notes regarding the samples (e.g. food type).

The Integrity of the data is also reported, as part of the 21CFR11 recommendations (see Appendix B).



**Fig. 53:** Information window

## 10 Creating templates

Templates allow the user to set-up runs that will be used repetitively. For example, you may want to run a group of assays using a particular set of controls and standards in specifically allocated wells all the time.

### 10.1 Create a template

**Start a New Run.**

Complete your run using the same methods described above.

**Select the down arrow next to the Save As button, then select Template.**

Save the template into the Template library located in  
*C:\ProgramData\R-Biopharm\RIDA®CYCLER\DefaultTemplates.*

You also have the option of creating subfolders within the library.

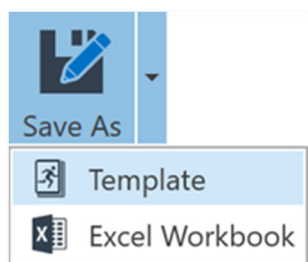


Fig. 54: **Save As** button

## 10.2 Opening a template

**Install Template (Help button on the tool bar).**

**In the **New** button on the tool bar, select the template.**

The run will open with all the saved parameters including sample annotation.  
Start the run as described above (section 9).

## 11 During a run

Once acquisition begins, the raw data is displayed for the channels selected under the **Data** section. The data is updated following each acquisition and the signal scaled to provide the best resolution for a group of samples, which can be, highlighted, or selected or deselected during a run. Analysis of data can be conducted prior to a run completing, if sufficient data is available to achieve meaningful analysis. Once the run has completed the raw data will always be available to view in future.

A **Run Summary** banner will appear as soon as the run commences.



Fig. 55: **Run Summary** banner

In the **Run Summary** banner, the hold temperature or cycle number is displayed to the left side of the banner next to the name of the instrument performing the run.

A graphic of the **Profile Summary** is also displayed. The section at which the run has progressed through is highlighted in grey.

The **Time Remaining** to completion of the run is displayed to the right side of the **Run Summary** banner.

### 11.1 Modifying a profile during a run (optional)

The **Run Summary** banner also has two functions to control the run: **Skip** and **Abort** a run.

#### **Skip a Section of the Profile**

Use the skip function if you wish to move to the next section of the profile.

You may choose to skip the last number of cycles of a run profile if you believe there has been a sufficient number of cycles to generate amplicon.

#### **Aborting a Run**

You can stop the run at any point by selecting the Abort function in the summary window.

**Warning, hot surface!**

In the event of a user aborted run do not open the lid until the instrument has cooled. The rotor within the chamber could be above 40 °C (104 °F). To avoid personal injury, do not touch the rotor for at least 5 min.

**11.2 Data**

The raw data is displayed for each channel being acquired and is listed in the **Navigator bar**. Cycle data is plotted as cycle number (x-axis) against the fluorescence value (y-axis) with a maximum fluorescence value of 100 units. At the start of the run the fluorescence will be scaled from 0 - 10 units or 70 - 90 units depending on the **Adjust Gain Settings** option chosen. As the real-time curve grows beyond 10 units or drops below 70 units, the graph is auto-scaled to ensure the maximum curve takes up 90 % of the visualized graph.

**11.3 Message**

Any warnings about the run will be displayed in Messages along with the time it occurred. Common messages will include the start time and instrument name and firmware version. Some messages may be warnings such as incompatibility of a selected assay or any loss in communication with the RIDA®CYCLER and when communication was restored. The **Autogain** values determined during the run will also be reported in the messages.

During analysis, all changes to parameters will be logged in the messages list once the run file is saved. This procedure allows the software to conform to 21CFR11, by creating audit trails (see Appendix B).

Messages				
Time	Priority	Category	User	Message
2/1/2018 3:47:28 PM	Information	Run	Admin	Run started via Serial on "RIDA Cyclex-365" S/N M0000365 F/W v2.24 S/W v0.1.0
2/1/2018 3:55:04 PM	Information	Run	Admin	Autogain completed for Green using Sample: 34, Detector gain: 6x, LED power: 303, Scale: 1.02
2/1/2018 3:55:06 PM	Information	Run	Admin	Autogain completed for Yellow using Sample: 34, Detector gain: 1x, LED power: 750, Scale: 1.35
2/1/2018 3:55:07 PM	Information	Run	Admin	Autogain completed for Red using Sample: 19, Detector gain: 1x, LED power: 400, Scale: 1.50

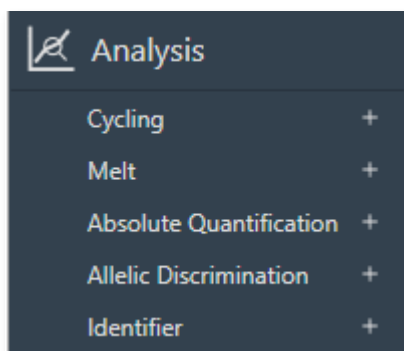
**Fig. 56:** Messages window**12 Analysis**

There are the following analysis types available in the software: **Cycling**, **Melt**, **Absolute Quantification**, **Allelic Discrimination** and **Identifier**. Analysis specific parameters are provided for edit, and report tables are displayed along with various graphs depending on the analysis type.

**To start a new analysis, select the **Add** button next to the analysis type in the navigator bar.**

Select from the following options:

1. Cycling Analysis
2. Melt
3. Absolute Quantification (includes Standard Curve)
4. Allelic Discrimination
5. Identifier



**Fig. 57:** Analysis bar

**Then select the target to analyze from the list of options.**

The targets listed will be based on the assays chosen before starting the run. For multiplex assays each target will be shown as part of the assay.

The target name selected will be displayed in the navigator bar below the analysis type. You may edit the name by double-clicking on it.

Multiple analyses are possible for each run, with the analysis being viewed highlighted blue on the navigator bar.

Delete any analysis by selecting the **Delete** button next to the target name.

## 12.1 Cycling analysis

**Cycling Analysis** determines the Cq value (quantification cycle) and reaction efficiency of each sample in the data set.

Cycling analysis is always provided with any other analysis type that uses cycling data (**Standard Curve** and **Absolute Quantification**), providing the option to adjust parameters associated with generating Cq and efficiency values.

By selecting **Cycling Analysis**, the software will, by default, plot baseline-corrected curves as fluorescence (y-axis) against cycle number (x-axis), in linear scale, for the target that was chosen.

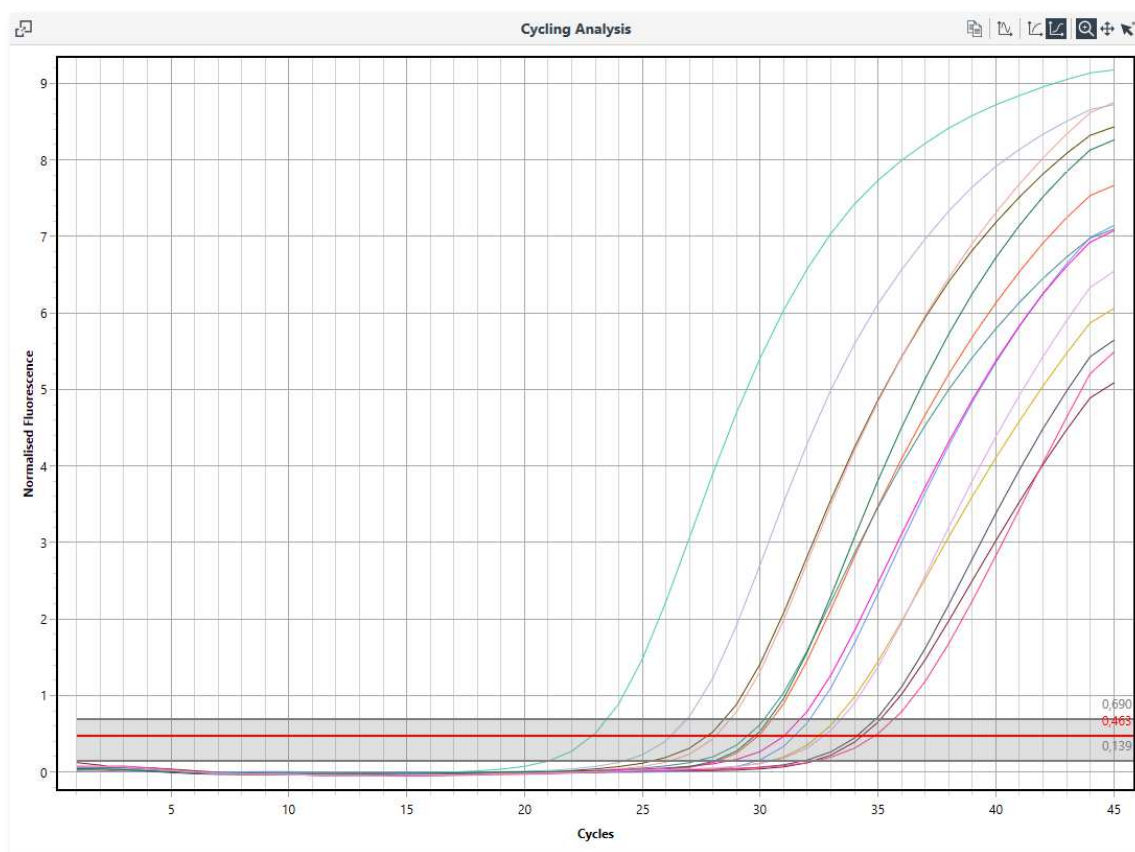


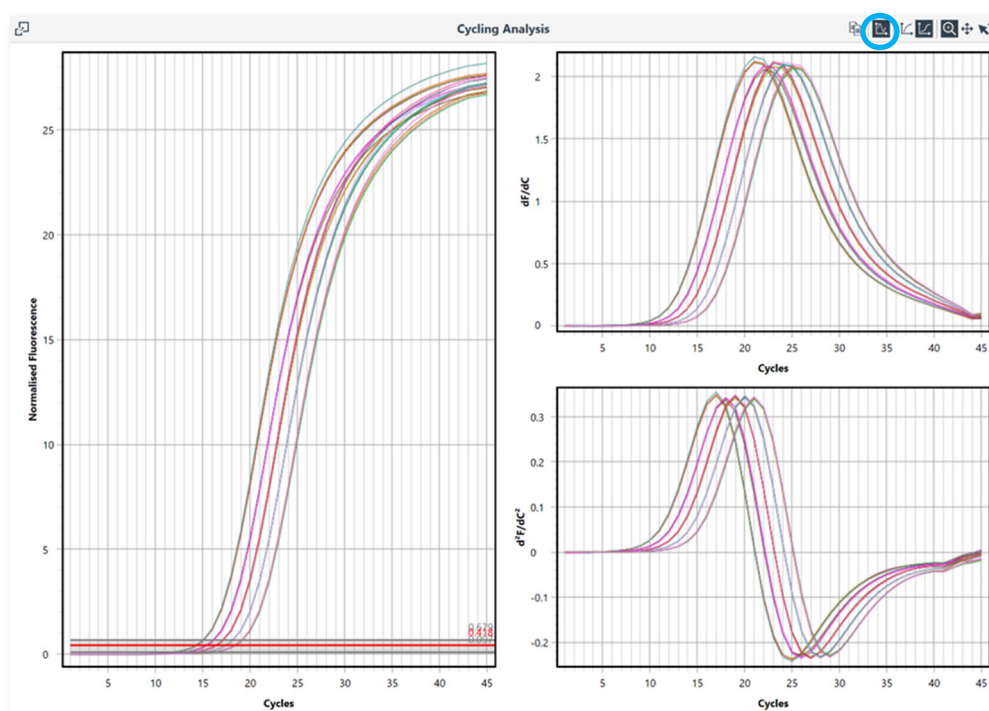
Fig. 58: Cycling Analysis window

### 12.1.1 Graph types



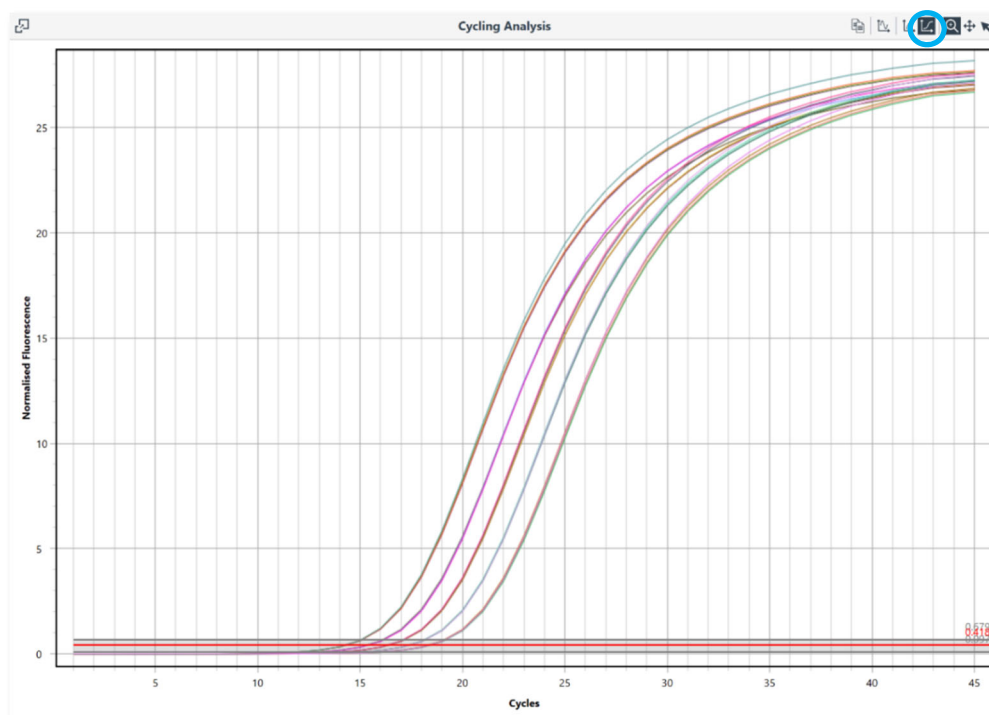
Fig. 59: Icon for toggling between different chart types

**Derivatives:** the first and second derivative curves for the selected data can be displayed in parallel with baseline corrected amplification curves.



**Fig. 60:** Cycling analysis options

**Linear y-axis:** display the baseline corrected cycling data with the y-axis in linear scale by selecting the Linear y-axis icon.



**Fig. 61:** Linear y-axis

**Log y-axis:** displaying the data in the logarithmic view allows you to better visualize the exponential region of the amplification curve and is therefore the default option. You can revert to the logarithmic scale by selecting the Log y-axis icon.

### 12.1.2 Cycling analysis parameter

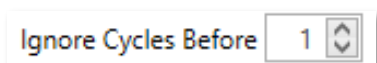
A default set of parameters will be applied to the assay data, providing automatic calculation of Cq and efficiency values, which are reported in the results table. You can change the following parameters: 'ignore cycles before', 'threshold start' and 'auto set threshold'.

#### Method

- **Dynamic:** determines the average baseline value measured prior to the detection of specific amplification (take-off), subtracts the average value from the measured values, then takes into account any slope in the baseline curve, to baseline correct the sample. The take-off is calculated by using the second derivative maximum as a starting point.

The dynamic algorithm tries to set a window of linearity (W-o-L, shaded gray in the diagram) by starting the upper limit of the window at the mean fluorescence level found at the maximum of the second derivative. Then, an iterative modification of the W-o-L is performed until the optimum is found with the smallest deviation between the individual efficiencies and the mean efficiency of the samples in the data set (Ramakers et al. 2003; Ruijter et al. 2009). From the W-o-L, the threshold (red line) is set to 75 % of the window area. Samples without detectable gain or samples below a user-defined fluorescence limit are not included in the W-o-L calculation.

**Ignore cycles before:** use this setting if there is a significant deviation in the baseline at the start of the run. These changes can occur due to many factors including too much template or insufficient denaturation of double stranded DNA. Applying this may improve analysis using **Dynamic** baseline correction.



**Fig. 62:** Ignore Cycles Before icon

**Cycle threshold:** The **cycle threshold** is used to determine the Cq value of each selected sample and will be set automatically by the software.

**Auto set threshold:** uses the W-o-L to set the cycle threshold.

For some data sets the W-o-L may not be determined and the threshold will be set to a default value of 0.1.

Both the W-o-L and threshold will change if samples are deselected or reinstated into the analysis, as the W-o-L is determined from the assay data set. If you wish to view specific samples without affecting the analysis, then use the **View** function in the **Samples Selector** to remove curves just from the graph rather than the analysis.



**Fig. 63:** Auto set threshold icon

## Manual adjustment of the threshold

To set the threshold manually, deactivate the **Auto set threshold** function. The desired threshold value can then be entered manually or set using the arrow keys.



The Parameters dialog box shows the following settings:

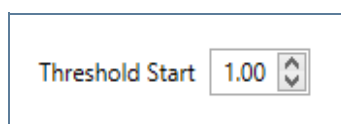
- Target: Salmonella spp Source Data: Cycling Green
- Method: Dynamic Ignore Cycles Before: 0
- Threshold Start: 1,00 Auto Set Threshold: ☒
- Exclusion: Extensive Fluorescence Cutoff Level: 5,0 %

**Fig. 64:** Input field for adjusting the threshold



For some data sets with poor amplification, a W-o-L may not be determined, resulting in an inability to set the threshold automatically. Under such circumstances a warning is displayed next to the Auto set threshold field.

**Threshold start:** you can avoid interfering parts at the start of the baseline-corrected real time curve by moving the threshold start position. This can be achieved by either entering the value in the Threshold start text box or moving your mouse left or right clicked on the green box at the start of the threshold line.



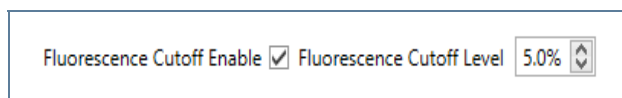
The Threshold Start input field shows the value 1.00 with up and down arrow buttons.

**Fig. 65:** Threshold Start icon



It is not recommended to analyze two different assays using the same cycle threshold or W-o-L. Different assays will have different efficiencies that can affect the performance of the analysis algorithms.

**Fluorescence Cutoff:** This parameter will exclude samples that fall below the defined percentage of the maximum fluorescence change. Small changes in fluorescence can interfere with the determination of Cq values by changing the W-o-L. Some of these small changes can be due to probe self-hydrolysis and cross talk between dyes. The default level is set to 5 %.



The Fluorescence Cutoff input field shows the value 5.0% with up and down arrow buttons.

**Fig. 66:** Fluorescence Cutoff Icon

**Auto Exclusion:** If the sample is excluded its Cq value is not determined and it is not included in the window of linearity calculation. Depending on the exclusion setting a sample may be excluded for one or more of the following reasons:

- **No amplification:**

The filtered fluorescence trace for the sample does not show sufficient amplification. The change in fluorescence for the sample must increase by 7 times. The criterion is taken from 'Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data' Nucleic Acids Research, 2009, Vol. 37, No. 6 e45, in the caption for Figure 3. To be included the sample fluorescence must satisfy the following condition:

$$f_{first} - f_{min} \geq 7 \times (f_{max} - f_{min})$$

where

$f_{min}$  is the smallest filtered fluorescence value;

$f_{first}$  is the first filtered fluorescence value different to  $f_{min}$ ;

$f_{max}$  is the maximum filtered fluorescence value.

- **Fluorescence Cutoff:**

The largest value for the filtered fluorescence trace for the sample does not exceed the fluorescence cut-off level and fluorescence cut-off is enabled.

- **Normalization failure:**

An initial linear region is unable to be found.

The exclusion is set to:

**Extensive:** samples will be excluded if they display no amplification, if normalization fails or they fail the fluorescence cut off.

If a sample is excluded its Result column entry of the Cycling Analysis Result table will be set to **Excluded**.

**Window of Linearity:** Any sample which is excluded will not be included in the window of linearity calculation. In addition, samples may also be excluded from the window of linearity calculation if:

- The second derivative maxima occur at the extreme of the data range;
- The logarithm of the normalized fluorescence trace is not defined near the second derivative maxima.

If a non-excluded sample was excluded from the window of linearity calculation, then it will not have an efficiency or  $R^2$  value calculation and its Result column entry of the Cycling Analysis Result table will be set to **Excluded from WOL**.

### 12.1.3 Cycling analysis results table

The results table is organized to display the mean ( $\bar{x}$ ) and standard deviation ( $x\sigma_{n-1}$ ) of the Cq values for sample replicates; and the individual sample results, which are organized, just below the replicate row, into the following columns:

Results				
Well	Cq	Efficiency	R <sup>2</sup>	Result
Standard 1			$\bar{x} = 14.29 \sigma = 0.02$	
1	14.31	0.91	1.00000	
2	14.29	0.92	1.00000	
3	14.31	0.91	0.99999	
4	14.27	0.92	1.00000	
Standard 2			$\bar{x} = 15.37 \sigma = 0.03$	
5	15.33	0.92	0.99999	
7	15.37	0.93	0.99998	
8	15.40	0.91	0.99998	
Standard 3			$\bar{x} = 16.37 \sigma = 0.03$	
Standard 4			$\bar{x} = 17.40 \sigma = 0.02$	
Standard 5			$\bar{x} = 18.38 \sigma = 0.03$	
Unknown Sample 2			$\bar{x} = 16.34 \sigma = 0.00$	

**Fig. 67:** Cycling analysis results table

**Well:** the order of the well numbers will depend on the grouping of the samples as the name of the sample order can be alphanumeric (grouped) or numeric (ungrouped).

**Cq:** the quantification cycle value for each sample, which is dependent on the cycle threshold set.

**Efficiency:** the amplification efficiency is calculated for each sample using the LinRegPCR algorithm described by Ramakers et al. (2003). Using the slope of the linear regression line from the calculated W-o-L, efficiency is calculated as;  $E = 10^{\text{slope}} - 1$ .

Alternatively, a single efficiency value can be determined for a selected assay using the Standard Curve Analysis method.

**R<sup>2</sup>:** the r-squared value is a quality measure of the linear regression used to calculate amplification efficiency (values > 0.98 are acceptable).

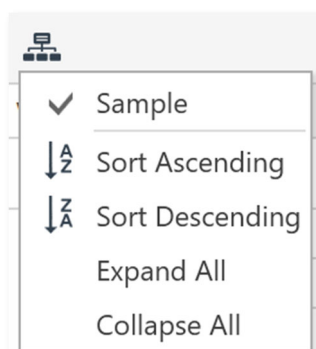
**Result:** any issue related to the quality of the sample is reported in the results column. For example, if a Cq value cannot be determined.

#### 12.1.4 Cycling analysis sample table selection

The result table can be compressed by hiding the individual sample results using the triangle at the top of each replicate row or selecting the **Full Collapse** option in the *Sample* table selector for the whole table. Left click the Sample table selector to view the different table sorting options.

To sort the samples in replicates using an alphanumeric order use the **Group by this column** option. Otherwise select the **Ungroup** option to have the samples displayed in order of well number.

The individual samples can be sorted in **Ascending or Descending** order for any of the columns for both grouped and ungrouped tables. Use this option to determine the range of Cq or efficiency values by displaying the highest to the lowest values.



**Fig. 68:** Cycling analysis sample table

### 12.1.5 Floating windows

Under some circumstances it may be necessary to view either the cycling analysis graph or cycling analysis parameters in parallel with another analysis type. For example, you may want to observe what a change in cycle threshold has to reaction efficiency using a standard curve. The floating window option allows you to achieve this multiple analysis window view.



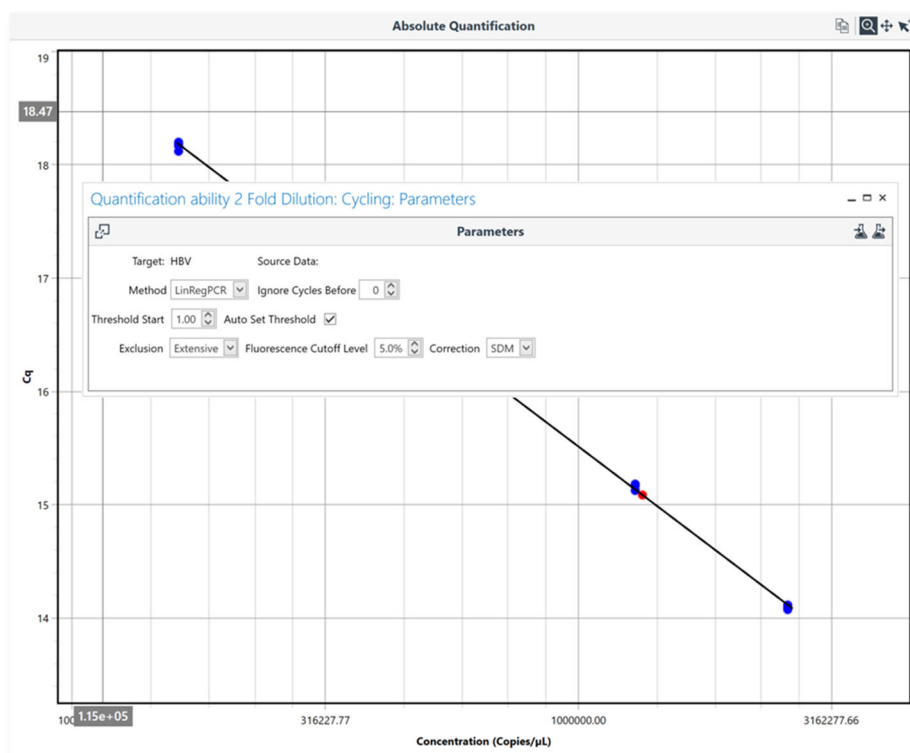
**Fig. 69:** Symbol for pop out copy of panel into floating window

#### **Select the Pop out copy of panel into floating window icon.**

Located in the top left hand corner of either the cycling analysis graph or parameters windows.

#### **You can move the floating window into any position of the screen.**

Once the window is floating you can select the parent analysis (e.g. Absolute Quantification). Any change made to the floating window will be represented in the associated parent analysis.



**Fig. 70:** Floating window

Select the delete icon in the top right corner of the floating window to close the floating window.

## 12.2 Melt

Melt Analysis allows you to determine the peak dissociation temperature ( $T_m$ ) of a sample from the melt data. Basic melt analysis can be typically used as a measure of analytical specificity for an assay, especially when using intercalating dyes, by detecting any non-specific amplicons such as primer dimers. Melt analysis can also be applied for the determination of genotypes using chemistries such as dual hybridization probes. Upon selecting the Melt Analysis option a graph will be displayed showing the first derivative curve plotted as  $\frac{dF}{dT}$  (y-axis) against temperature ( $^{\circ}\text{C}$ , x-axis), for the target that was chosen. The melt curve threshold can be set to any value, along with various other melting parameters available for genotyping.

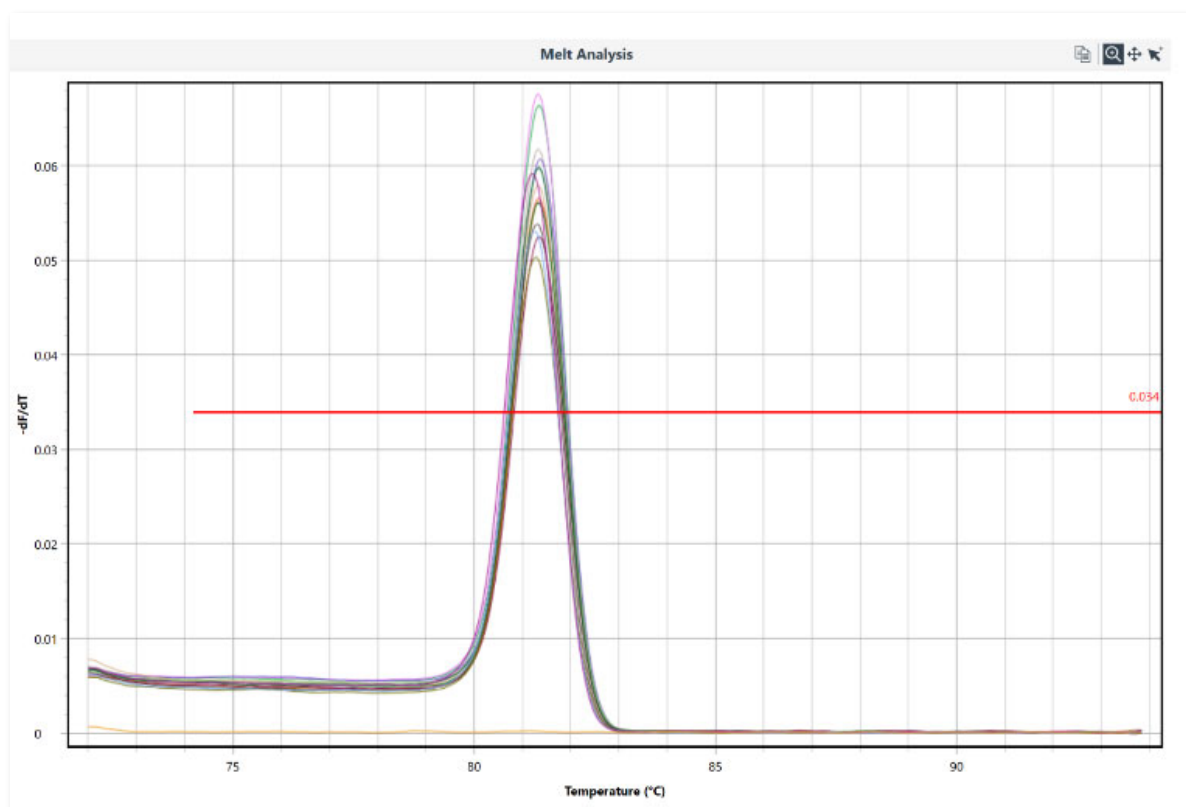


Fig. 71: Melt analysis window

### 12.2.1 Melt analysis parameter

The following parameter can be changed:

Target: IC/Dientamoeba fragilis

Threshold  starting at  °C

Invert Data ☐

Filtering

Fig. 72: Adjustable parameters in melt analysis

**Threshold:** change the threshold level by either sliding the red line up or down on the graph; or by entering a numerical value in the **Threshold level** text box. Only peaks above the threshold line will be reported.

**Threshold Start:** to ignore earlier peaks, slide the red line from left to right by clicking on the green box at the start of the threshold line, or enter a numerical value in the **Threshold Start Temperature** text box.

**Invert:** the first derivative melt curves can be inverted to allow for analysis of data generated using chemistries such as quenched FRET dual hybridization probes or Plexor®. Tick the **Invert** box to invert the melt curves.

## 12.3 Absolute Quantification

This analysis setting allows for the absolute quantification of unknown samples using a standard curve. Furthermore, together with a reference material of known concentration of allergens the content of unknown samples can be characterized with units like e.g. ppm.

### 12.3.1 Quantification analysis using standard curves

The Standard Curve Analysis feature allows you to determine the efficiency of an assay by using a serial dilution series of known sample. This method can be used as an alternative to the LinRegPCR method in calculating reaction efficiency. The analysis method is located within the Absolute Quantification analysis option.

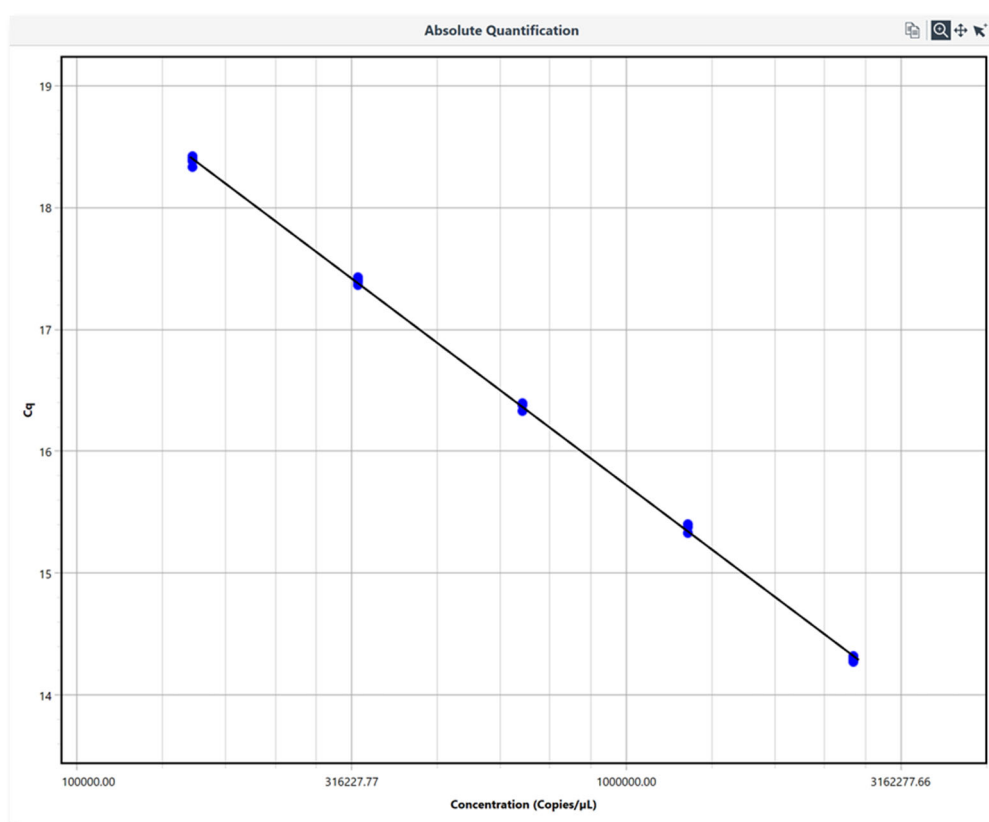
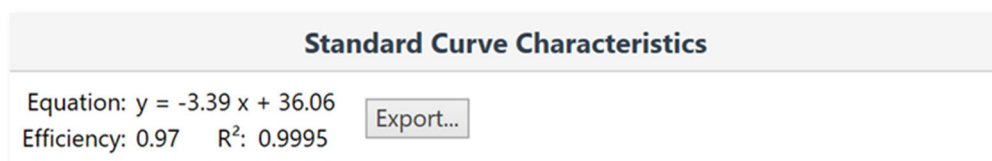


Fig. 73: Standard curve - data points of the serial diluted standard DNA are shown in blue.

### 12.3.2 Standard curve characteristics

The method utilizes the Cycling Analysis feature to determine Cq values and plots them (y-axis) against the log of the given concentration (x-axis) for each standard annotated in the Samples editor. A line of best fit is generated for the data plot from which the slope of the line is determined.

**Efficiency:** From the slope of the line the efficiency is calculated using the equation  $10^{\frac{-1}{\text{Slope}}} - 1$ , and is reported in a summary window as a value from 0 to 1 along with the R-squared ( $R^2$ ) value, Equation of the line, which includes the gradient of the line ( $M$ ), and y-intercept.



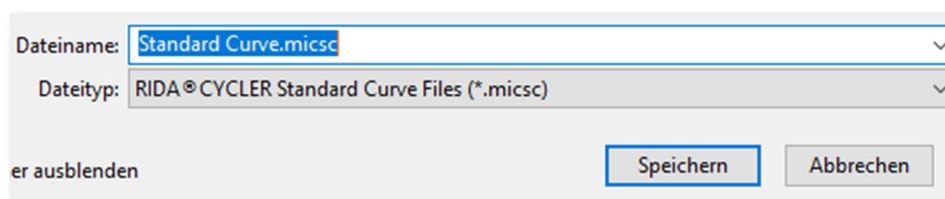
**Fig. 74:** Standard Curve Characteristics

**R-squared Value:** The  $R^2$  value is a measure of the percentage of data that matches the hypothesis that the given standards form a standard curve. In other words, if the  $R^2$  value is low then the given standards do not aggregate to the line of best fit very well, and therefore, the calculated efficiency may not be reliable.

A value  $> 0.98$  is typically a good  $R^2$  value. However, a good  $R^2$  value can still be achieved for a poor standard curve if not enough standards have been used. It is recommended that the standards extend to at least 5  $\log_{10}$  concentrations.

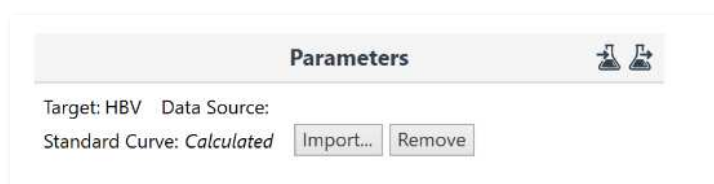
**Export the standard curve in order to reuse it in subsequent experiments.**

The standard curve is saved into a file location as a \*.micsc file. This file can be imported into any run containing cycling data and used as part of the Absolute Quantification analysis.



**Fig. 75:** Exporting the standard curve

### 12.3.3 Importing a standard curve



**Fig. 76:** Parameters Import of a standard curve

**Import a standard curve.** Locate the standard curve file (\*.micsc) in the location the standard curve was exported to. The software will apply the standard curve formula to the run data and display the curve on the graph.

**It is required that a standard curve calibrator be used to verify the imported standard curve.** In the Samples editor annotate the calibrator by giving it a Type, Standard. Then enter the concentration for the standard calibrator. A number of replicates can be run for a calibrator. Any number of calibrators can be used with an imported curve. Failure to apply a standard calibrator will result in a warning being displayed above the standard curve graph. A warning will also be displayed if the standard calibrator varies in  $C_q$  by more than  $\pm 1$  cycles.

### 12.3.4 Standard curves results table

The **Standard Curve Results table** contains the following measures:

**Cq:** the quantification cycle value for an individual standard.

**Given Concentration:** the given concentration is the value annotated in the **Samples editor**. The reported units are also displayed in the column heading. The unit measure is selected from a list in the **Samples editor**.

**Calculated Concentration:** is an adjusted concentration for an individual standard based on the line of best fit. The adjusted concentration is calculated by using the Cq value to interpolate the new concentration from the line of best fit. The mean ( $\bar{x}$ ) and standard deviation ( $\sigma$ ) for a set of replicates is also provided in the top row for the set.

**Percentage Variation:** is the percentage difference between the given and calculated concentrations.

Standard Curve Results						
Well	Sample	Cq	Given Concentration (Copies/Reaction)	Calculated Concentration (Copies/Reaction)	% Variation	Calculated Reference (ppm)
12	S0	19,52	5E+05	5,318E+05	6,37	278,5
13	S1	22,74	5E+04	5,329E+04	6,57	27,9
14	S2	26,27	5000	4282	14,35	2,242
15	S3	29,45	500	439,9	12,02	0,2303
16	S4	32,28	50	58,54	17,07	0,03065

Fig. 77: Standard curve results table

### 12.3.5 Cycling analysis for standard curves

**Cycling Analysis** is used to determine the Cq values for the standard curve. Therefore, **Cycling Analysis** is automatically paired with, and will appear beneath, the **Standard Curve Analysis** in the navigator bar. Use **Cycling Analysis** to make modifications to analysis parameters such as **Threshold Start** or **Ignore Cycles before**. The same assay parameters as defaulted for an individual **Cycling Analysis** will be applied when paired with **Standard Curve Analysis**.

Using the Samples selector to remove samples from the **Cycling Analysis**, will result in a recalculation of the amplification efficiency in the **Standard Curve Analysis**.

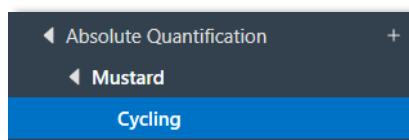
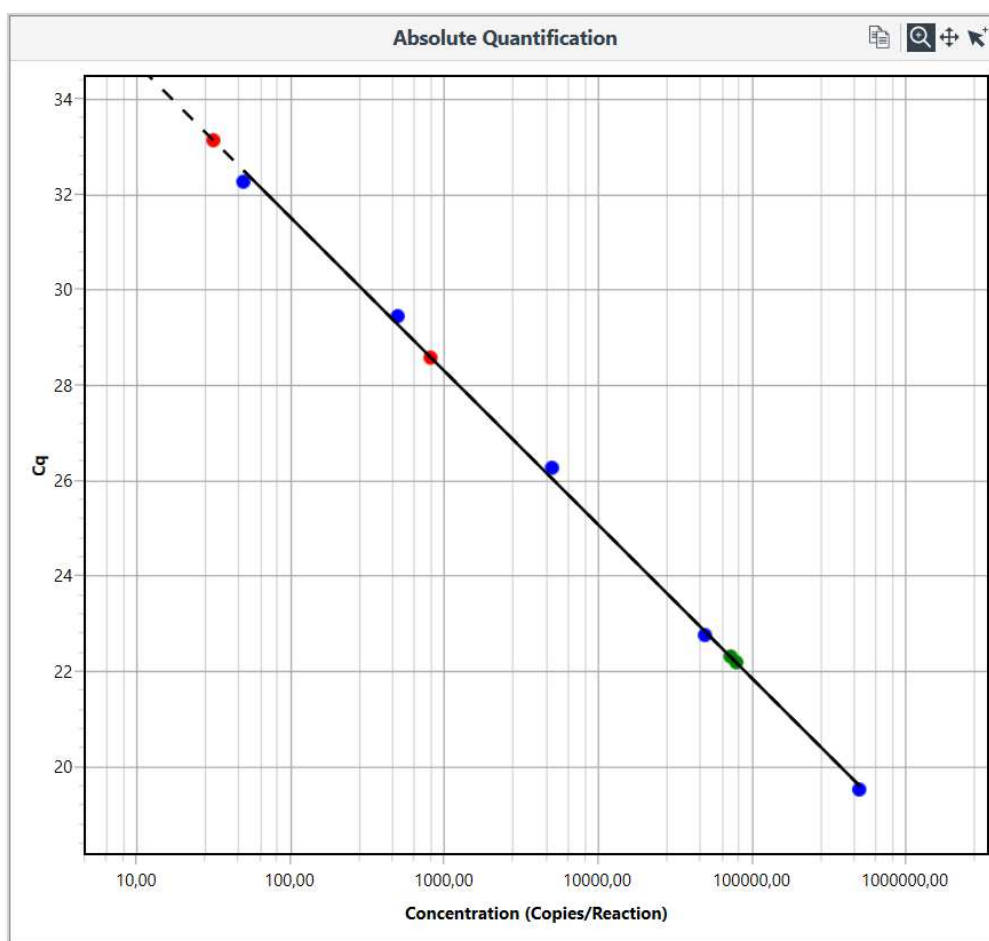


Fig. 78: Absolute quantification navigator bar

### 12.3.6 Absolute quantification using reference material

The **Absolute Quantification** analysis method shares all of the features mentioned above in the section **Standard Curve Analysis** but with the addition of the determination of unknown concentrations using reference material.

In the graph for the standard curve, standards are shown as **blue** dots and unknowns as **red** dots. Reference material is shown in **green**. Samples that fit outside the limits of the standard curve will be displayed on a dashed line. Samples outside the limits of a standard curve should be treated with caution. To avoid having samples outside the limits of the standard curve, ensure that your standard curve contains enough data points to encompass all of your potential unknowns. This may require the determination of the linear dynamic range and/or limit of detection (LoD) for the assay.



**Fig. 79:** Standard curve graph - standards marked blue, unknown samples marked red, reference material shown in green

### 12.3.7 Sample results table

The **Sample Results table** displays the **Calculated Concentration** for each unknown sample. The mean and standard deviation for a set of replicates is also provided in the top row for the set. The reported units are displayed under the **Calculated Concentration** title and are chosen from a list in the **Samples editor**. The Cq values for each sample are also reported in the **Sample Results table**.

Sample Results					
Well	Sample	Cq	Calculated Concentration (Copies/Reaction)	Calculated Reference (ppm)	
1	Sample 1	34,05	16,53	0,008658	^
2	Sample 2	35,16	7,478	0,003915	
3	Sample 3	35,46	6,011	0,003147	
4	Sample 4	32,72	42,56	0,02228	
5	Sample 5	33,14	31,63	0,01656	
6	Sample 6	32,61	46,09	0,02413	
7	Q40	22,30	7,308E+04	38,27	
8	Q40	22,17	7,985E+04	41,81	
9	EC				
10	NTC				
11	PTC	28,58	819,4	0,429	v

**Fig. 80:** Sample results table

## 12.4 Allelic Discrimination

Allelic discrimination allows for the determination of genotypes using real time kinetic data obtained from multiplexed assays using hydrolysis probe chemistry. The presence of an allele is indicated by a real-time amplification curve within the specific channel corresponding to the probe designed toward it. The presence of both alleles will indicate heterozygosity.

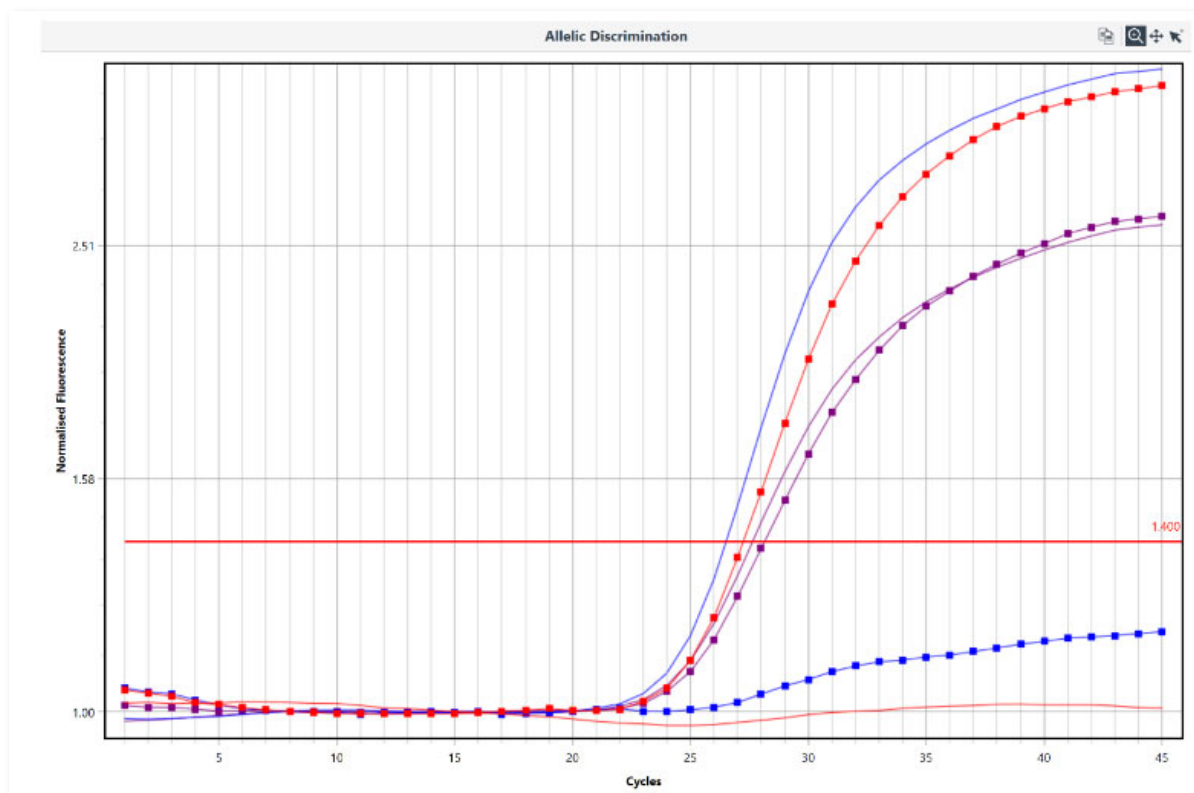


Fig. 81: Allelic discrimination window

### 12.4.1 Allelic discrimination parameters

Manually set the Threshold to a level required to distinguish each allele from background.

### 12.4.2 Allelic discrimination results table

Once the genotype names have been linked to each allele and the threshold is set, the samples are called in the Results table.



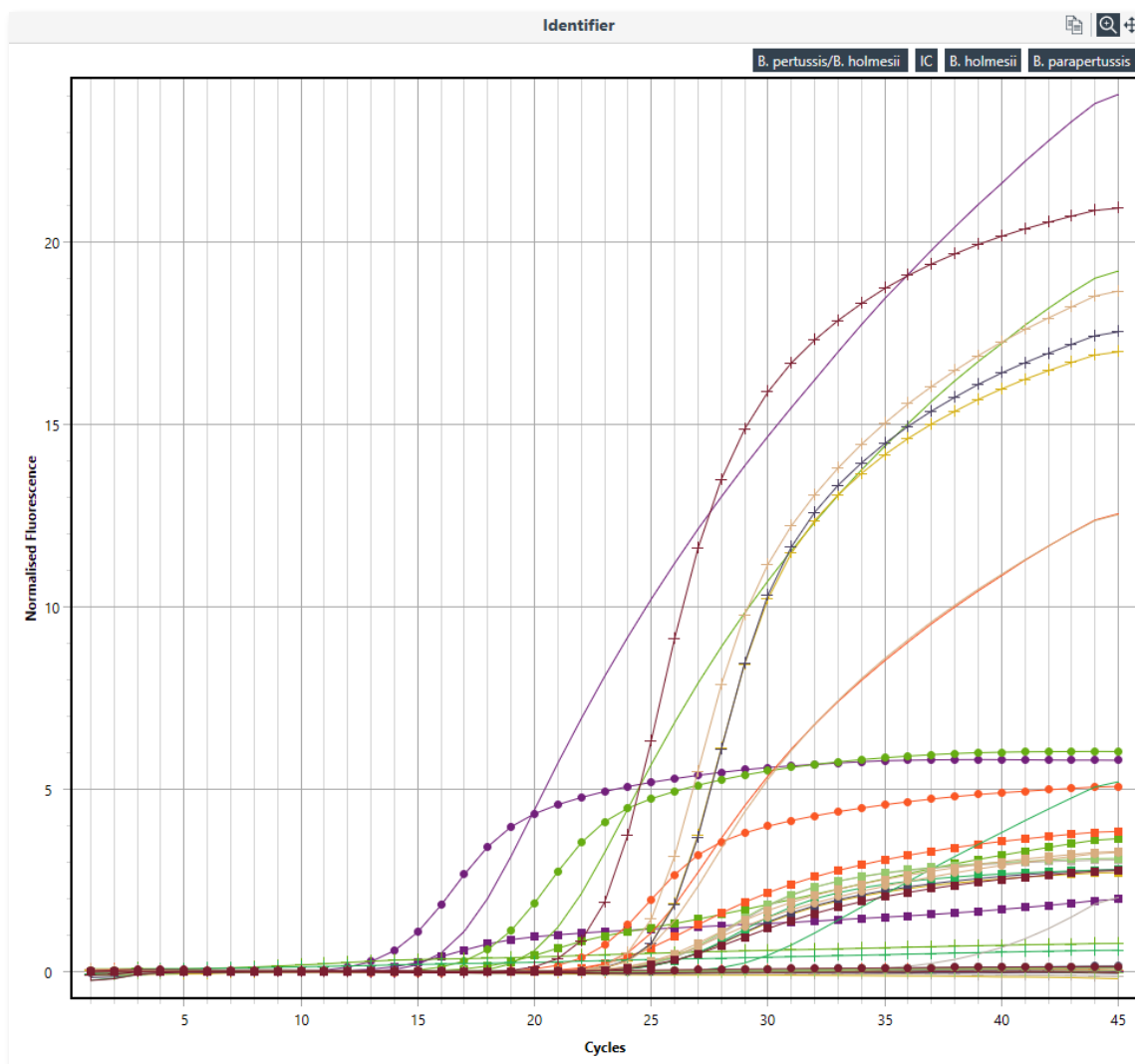
Results  		
Well	Sample	Genotype
1	Sample 1	Heterozygote
3	Sample 2	Wild Type
8	Sample 3	Mutation

Fig. 82: Sample result table

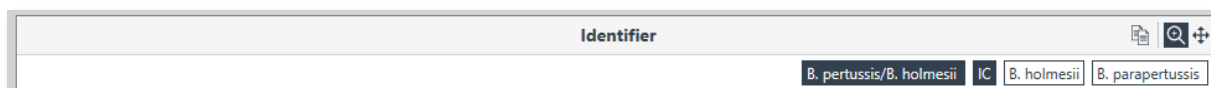
## 12.5 Identifier

Normalized real-time PCR curves are shown for each target in the analysis graph. Curves are displayed in linear format. You have the option to display all of the targets on the same graph or on individual graphs using the Chart Display icons. When the targets are displayed individually, selection of the required target is achieved using the chart tabs.



**Fig. 83:** Identifier window

The individual channels can be selected or deselected via the channel list in the upper right part of the identifier.



**Fig. 84:** Setting of the channel view

## 13 Projects

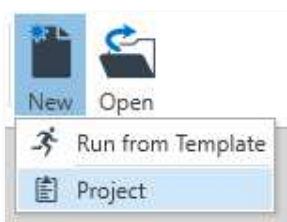
Projects allow the user to combine multiple runs into one analysis. Up to ten runs can be combined per project allowing up to 480 samples to be analyzed at once.



### Safety information!

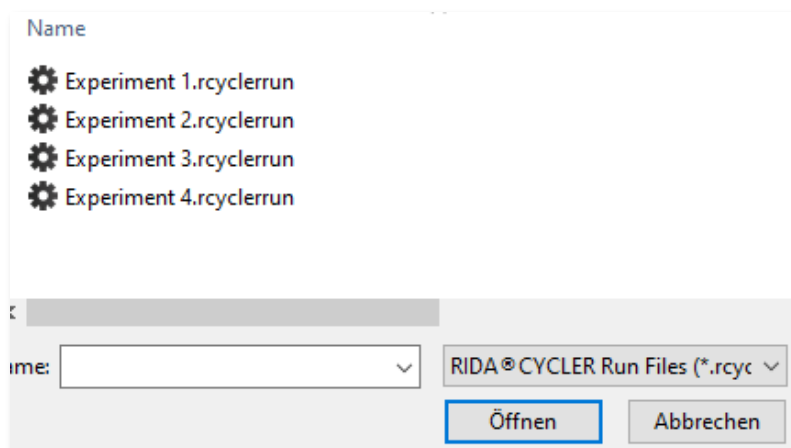
The current version (1.2.) only allows for Cycling Analysis, Melt Analysis and Absolute Quantification.

To analyze multiple runs, select **New** and then Projects.



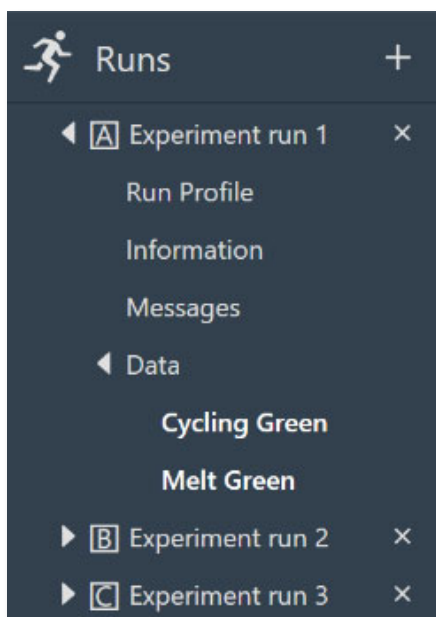
**Fig. 85:** Project creation

Via **Runs +** button in the **Run Navigator** the computer can be browsed for run files. Select the run files you wish to combine and analyze.



**Fig. 86:** Selection of run files

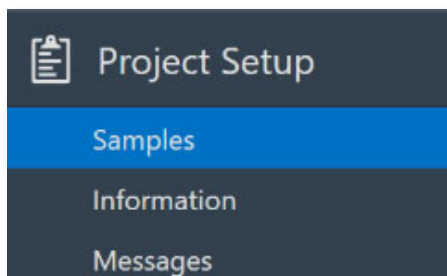
Ensure that the run files are compatible with each other. For example, make sure that the run profiles match. Each added run file will be labelled alphabetically (A, B, C...) in the order they are selected. Samples from that run will also be labelled with the run prefix in the results table. Information about each run file, such as Profile, Samples, and the raw Data, can be brought up by opening the run file tree in the Navigator bar. Remove a run file from the project by using the **Delete** button.



**Fig. 87:** Retrieving data for individual runs

Each Assay used in the run set will appear in the Assay section of the Navigator bar. You can look at the Assay settings by opening up the tree.

View and/or modify the **Samples** Edit or through the **Project Setup** section of the Navigator bar.



**Fig. 88:** Changing samples in project setup

Samples are displayed for each run and are listed in the order that the run was selected at the start. The run name is displayed at the top of the sample list and each run list can be collapsed or expanded using the small triangle icon to the left of the run name. Well-number styles are retained until you select the format in the project sample editor where all runs are reset to that format. The selected button in the sample editor will be set to the option used by the first run, so if you want to change all runs to match, you have to select another option then change back to the one required.

Samples

Search





















AA  
AA  
AA

A1  
A2  
A3

1..  
48

A1..  
H6

A7..  
H12

	Colour	Name	Type	Standards Concentration Copies/μL	Gr
	▶ Run Name: Experiment run 1				
	▶ Run Name: Experiment run 2				
	◀ Run Name: Experiment run 3				
1		Standard 1	Standard	50000000	
2		Standard 1	Standard	50000000	
3		Standard 1	Standard	50000000	
4		Standard 2	Standard	12500000	
5		Standard 2	Standard	12500000	
6		Standard 2	Standard	12500000	
7		Standard 3	Standard	781250	
8		Standard 3	Standard	3125000	
9		Standard 3	Standard	3125000	
10		Standard 4	Standard	3125000	
11		Standard 4	Standard	781250	
12		Standard 4	Standard	781250	
13		Standard 5	Standard	195000	
14		Standard 5	Standard	195000	
15		Standard 5	Standard	195000	
16		Sample 1	Unknown		
17		Sample 1	Unknown		
18		Sample 1	Unknown		
19		Sample 1	Unknown		
20		Sample 2	Unknown		

**Fig. 89:** Sample overview

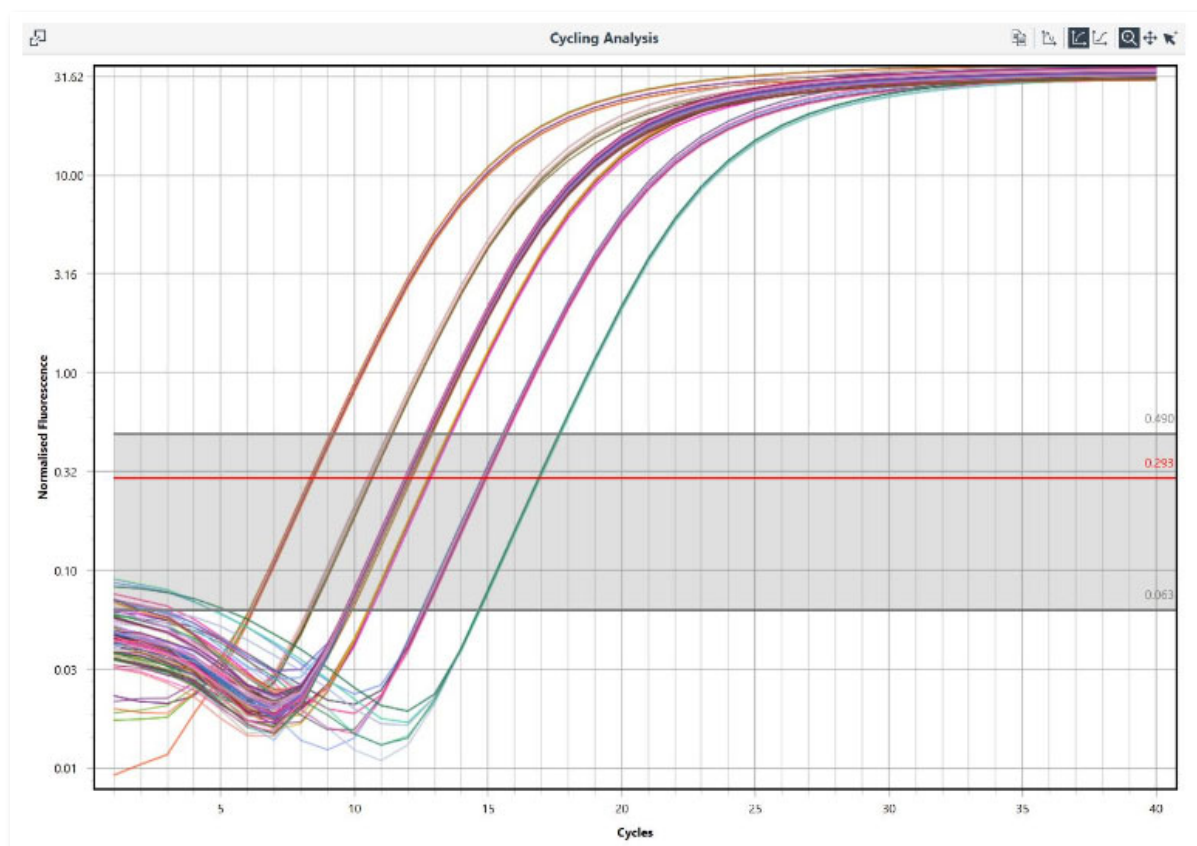
Enter any Information about the project in the field provided.

All operations during the analysis of the project will be recorded and displayed in the **Messages** section.

### 13.1 Project analysis

Select the + icon next to analysis type required, to start a new analysis. Chose the assay you wish to analyze from the list of options provided.

All the samples will be displayed in the one graph.



**Fig. 90:** Display of all samples in one graph

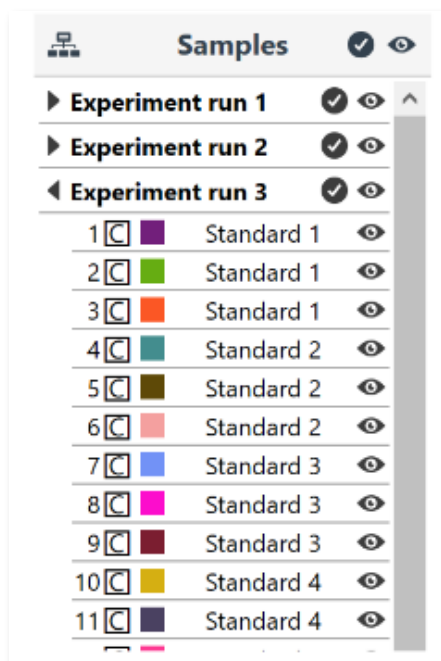
All of the same options available for each analysis type are available for a **Project** (see Analysis).

The result tables are similar, however, the results for individual samples are reported with a run order pre-fix for the well (e.g. 1A, 1B, 1C...).

Results				
Well	Cq	Efficiency	R <sup>2</sup>	Result
▶ NTC				
▶ Sample 1		$\bar{x} = 12.00 \sigma = 0.07$		
16[A]	11.93	0.99	0.99996	
16[B]	11.95	0.99	0.99995	
16[C]	11.95	0.99	0.99995	
17[A]	11.97	0.98	0.99996	
17[B]	12.12	0.99	0.99999	
17[C]	12.12	0.99	0.99999	
18[A]	12.00	0.98	0.99995	
18[B]	12.02	0.98	0.99994	
18[C]	12.02	0.98	0.99994	
19[A]	11.91	0.98	0.99994	
19[B]	12.03	0.99	0.99997	
19[C]	12.03	0.99	0.99997	
▶ Sample 2		$\bar{x} = 11.96 \sigma = 0.02$		
▶ Sample 3		$\bar{x} = 11.95 \sigma = 0.06$		
▶ Sample 4		$\bar{x} = 11.95 \sigma = 0.07$		
▶ Sample 5		$\bar{x} = 11.95 \sigma = 0.05$		
▶ Sample 6		$\bar{x} = 11.94 \sigma = 0.04$		
▶ Sample 7		$\bar{x} = 11.95 \sigma = 0.04$		
▶ Sample 8		$\bar{x} = 11.94 \sigma = 0.06$		
▶ Standard 1		$\bar{x} = 8.35 \sigma = 0.06$		
▶ Standard 2		$\bar{x} = 10.51 \sigma = 0.06$		
▶ Standard 3		$\bar{x} = 13.43 \sigma = 0.97$		
▶ Standard 4		$\bar{x} = 14.14 \sigma = 1.03$		
▶ Standard 5		$\bar{x} = 16.89 \sigma = 0.03$		

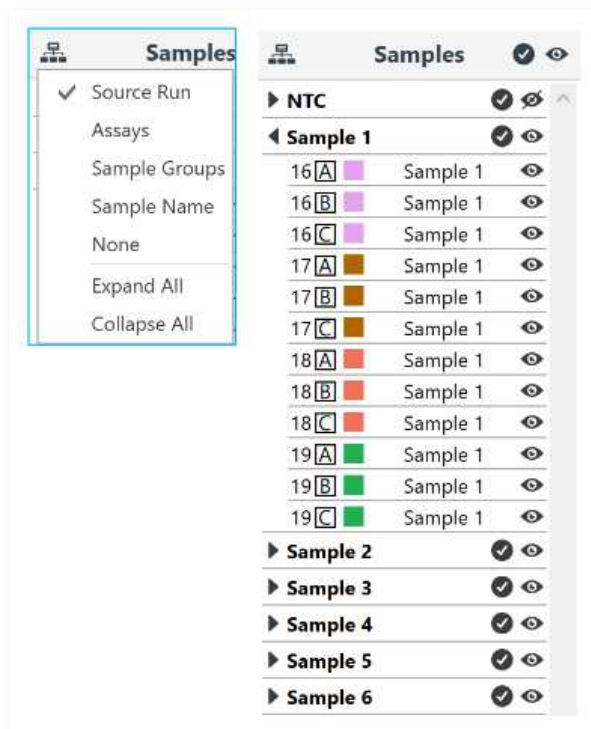
**Fig. 91:** Sample result table

The default view for the Samples Selector is divided into runs, with the run name displayed above the list of samples. These can be expanded or collapsed using the small triangle icon.



**Fig. 92:** Samples Selector

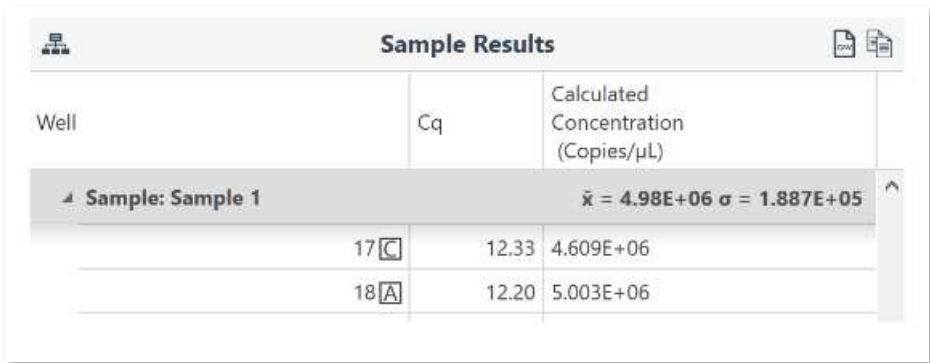
The **Samples Selector** can also be instructed to show the samples in order of **Assays**, **Sample Groups**, **Sample Name**, or **None** (well order). The groups can also be expanded or collapsed within the Samples Selector using the grouping function.



**Fig. 93:** Setting and sorting of samples in the **Samples Selector**

### 13.2 Concentration Settings for Projects (via analysis option “Absolute Quantification”)

Single concentration vs per-channel concentration setting is taken from the first run imported. If concentration units are different they are changed to "Mixed" and the user has to resolve it themselves.



Well	Cq	Calculated Concentration (Copies/μL)
Sample: Sample 1 $\bar{x} = 4.98E+06$ $\sigma = 1.887E+05$		
17 <input type="checkbox"/>	12.33	4.609E+06
18 <input checked="" type="checkbox"/>	12.20	5.003E+06

Fig. 94: Adjustment of concentration

Projects can be saved into any file directory.

### 13.3 Amplitude correction for Cycling Analysis

To correct for variations in signal amplitude for positive samples between runs and instruments, the software uses an amplitude correction algorithm.

To ensure amplitude correction is applied during cycling analysis you must ensure that the same sample is added between different runs. The sample, called an Amplitude Corrector, can be an unknown, standard or positive control. That is any sample that will generate a signal with an observable amplitude. NTC, NRT and negative controls should not be used. Ensure that the same name in the Sample Editor is used between the different runs. Multiple samples can be used, but you must ensure a link exists between each of the different runs being analyzed as part of a project. For example: Sample 1 could be used between runs A and B, and Sample 95 could be used between runs B and C.

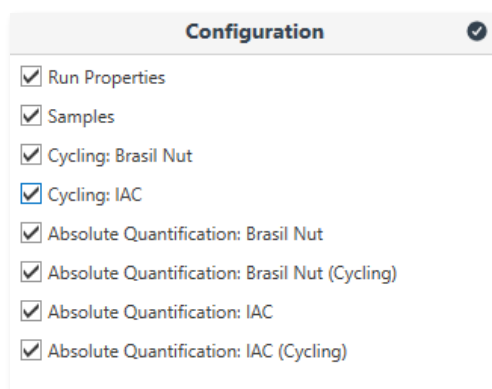
Amplitude correction works by using the First Derivative Maximum to determine a scaling factor between the different runs using the amplitude corrector samples. The scaling factor is then applied to all samples to correct for amplitude differences observed between runs or instruments.

## 14 Reports

Generate a report for each Run using the Reports + button in the Run Navigator. A standard report will be displayed containing information about Run Properties, Samples, and Analysis. A preview of the report will be displayed to the right and can be configured to show only certain parts of the run.

### 14.1 Report configuration

Each report is divided into two standard sections; Run Properties and Samples. The remaining sections will depend on the Analysis selected in the run. You can choose which sections to display in the report by ticking or unticking the sections in the report Configuration.



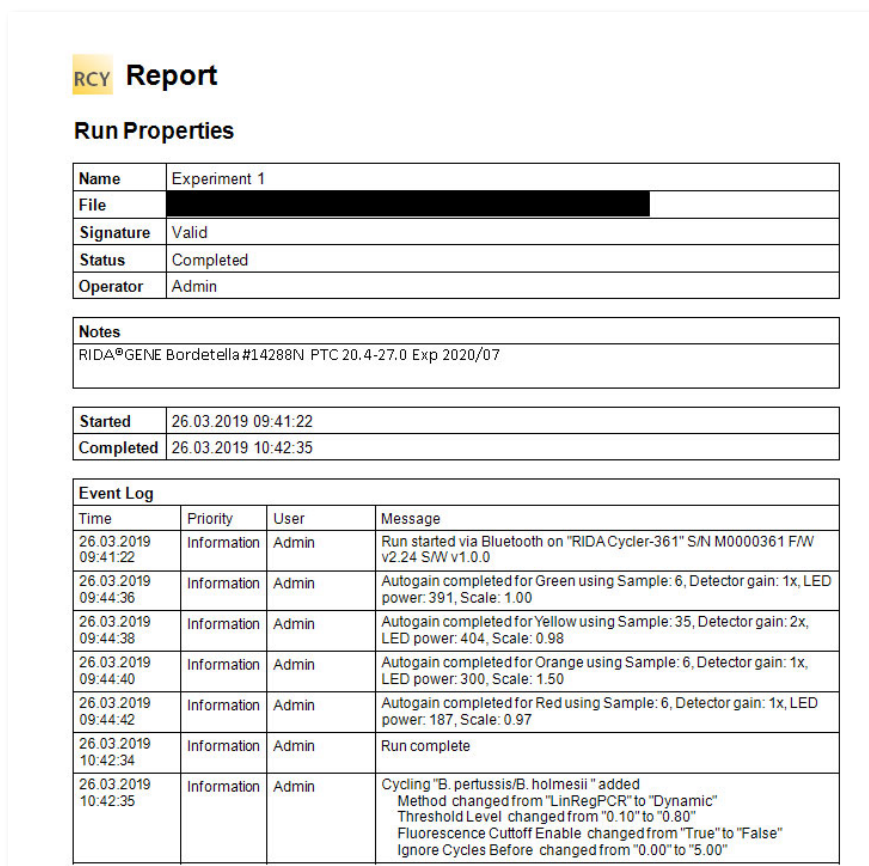
**Configuration**

- ☒ Run Properties
- ☒ Samples
- ☒ Cycling: Brasil Nut
- ☒ Cycling: IAC
- ☒ Absolute Quantification: Brasil Nut
- ☒ Absolute Quantification: Brasil Nut (Cycling)
- ☒ Absolute Quantification: IAC
- ☒ Absolute Quantification: IAC (Cycling)

**Fig. 95:** Report configuration options, certain parts can be selected or unselected by check

## 14.2 Report preview

Each selected section will be displayed in the report **Preview**. A new page will begin following each section. Each page will have a number in page footer along with the version of software used. The run name will be displayed in the page header.



**RCY Report**

**Run Properties**

<b>Name</b>	Experiment 1
<b>File</b>	[REDACTED]
<b>Signature</b>	Valid
<b>Status</b>	Completed
<b>Operator</b>	Admin

**Notes**

RIDA®GENE Bordetella #14288N PTC 20.4-27.0 Exp 2020/07

<b>Started</b>	26.03.2019 09:41:22
<b>Completed</b>	26.03.2019 10:42:35

**Event Log**

Time	Priority	User	Message
26.03.2019 09:41:22	Information	Admin	Run started via Bluetooth on "RIDA Cyclyer-361" S/N M0000361 F/W v2.24 S/W v1.0.0
26.03.2019 09:44:36	Information	Admin	Autogain completed for Green using Sample: 6, Detector gain: 1x, LED power: 391, Scale: 1.00
26.03.2019 09:44:38	Information	Admin	Autogain completed for Yellow using Sample: 35, Detector gain: 2x, LED power: 404, Scale: 0.98
26.03.2019 09:44:40	Information	Admin	Autogain completed for Orange using Sample: 6, Detector gain: 1x, LED power: 300, Scale: 1.50
26.03.2019 09:44:42	Information	Admin	Autogain completed for Red using Sample: 6, Detector gain: 1x, LED power: 187, Scale: 0.97
26.03.2019 10:42:34	Information	Admin	Run complete
26.03.2019 10:42:35	Information	Admin	Cycling "B. pertussis/B. holmesii" added Method changed from "LinRegPCR" to "Dynamic" Threshold Level changed from "0.10" to "0.80" Fluorescence Cutoff Enable changed from "True" to "False" Ignore Cycles Before changed from "0.00" to "5.00"

**Fig. 96:** Report preview

### 14.2.1 Run properties

The **Run Properties** section will display the following:

<b>Name</b>	The name of the run, given by the user.
<b>File</b>	The directory location of the run file.
<b>Status</b>	Valid or invalid
<b>Operator</b>	Name of the individual that completed the run. This can be adjusted on <i>Run Setup/Information</i> .
<b>Notes</b>	Any notes regarding the run completed by the user (on <i>Run Setup/Information</i> )
<b>Started</b>	Date and time run began (YYYY-MM-DD HH:MM:SS)
<b>Completed</b>	Date and time run finished (YYYY-MM-DD HH:MM:SS)
<b>Event Log</b>	A report of important messages generated during the run including any issues such as loss of communication.

### 14.2.2 Samples

The **Samples editor** is replicated in the report preview including sample **Name**, **Type**, standard concentrations, and **Assay**. All 48 samples will be displayed.

### 14.2.3 Analysis

Each analysis will be displayed depending on the information provided for the analysis. Only the targets selected will be displayed. To display multiple targets, open up a new analysis for the next targets required. The analysis parameters are displayed at the top of the page followed by a graph of the results. Finally, the results table is reported below the analysis graph.

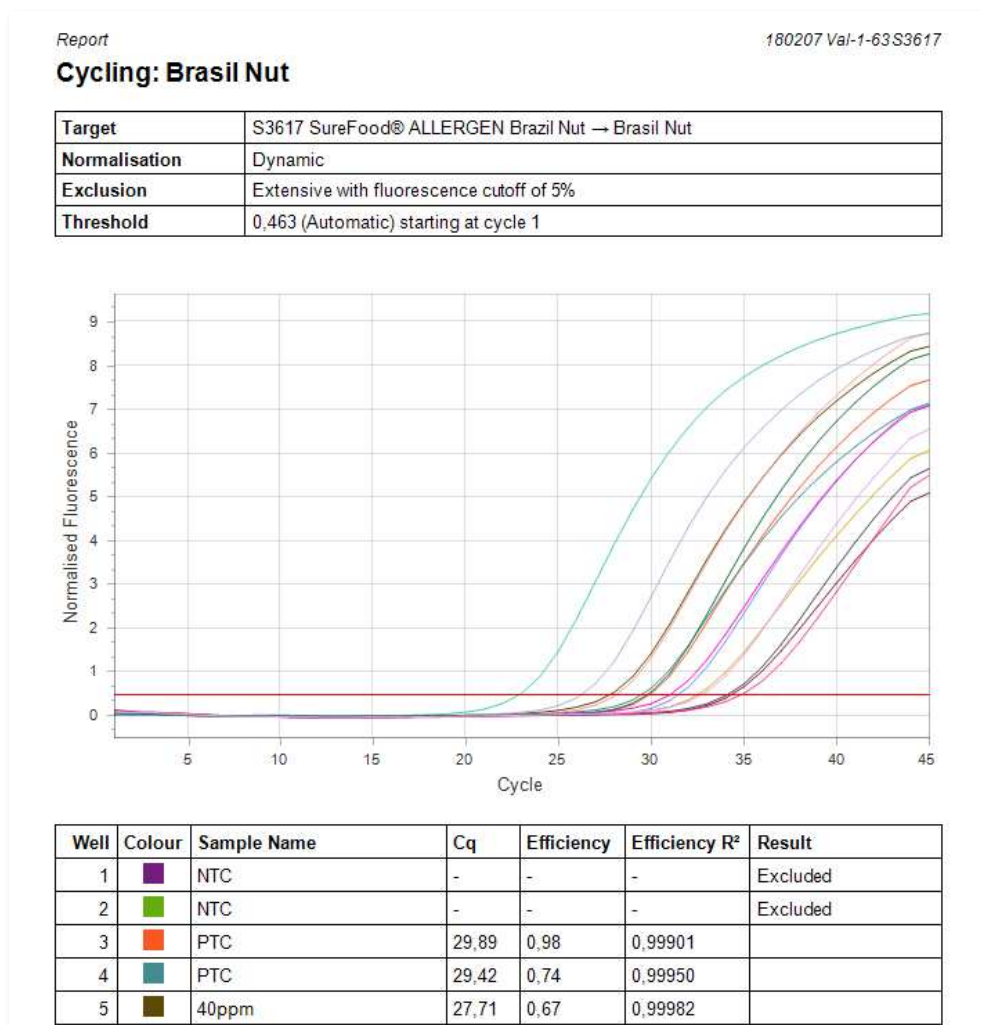


Fig. 97: Example for a report analysis

### 14.3 Report options



Fig. 98: Menu bar with further report options

<b>Search</b>	Find a word or string of characters in the report preview. Enter the search word(s) to find them in the report. The located words will be highlighted in blue in the preview and can be viewed one after the other with the Enter key. For this document search there are two more options which can be selected via Settings next to the input field (Whole Words Only and Case Sensitive).
<b>Print</b>	Print the report using user defined settings.
<b>Quick Print</b>	Print the report using default print settings.
<b>Page Setup</b>	Select the paper type and orientation and adjust page margins.
<b>Page Selection</b>	Navigate through the pages using the page selection buttons; <b>First page</b> ,

	<a href="#">Previous page</a> , <a href="#">Next page</a> and <a href="#">Last page</a> .
<b>Zoom</b>	Use the zoom in or zoom out to best view the report preview.
<b>Export</b>	Export the report using one of the available file formats including PDF, XLS and Text file. Each export will have a set of options to choose from.
<b>Send</b>	Email a report using one of the available file formats. A report generated in the selected file format will be attached to an email using your default email client, which will open automatically (if available).
<b>Watermark</b>	Add a watermark to your report. The water mark can be either a text or image. This option can be used to embed text such as <i>Confidential</i> to the report. A list of default text is provided or you can enter your own. Alternatively, add an image to the report such as a company logo. The direction and position of the text or image can be configured as well as which pages to apply the watermark to.

## 15 Excel options

Convert a run file into an Excel workbook with worksheets for each section. Do the following to create an excel workbook from your run file:

Click on **Save As** then select **Excel Workbook (\*.xlsx)** from the **Save As Type** options.

Once the Excel Workbook is created, open it to view the run file.

The workbook will be divided into the following worksheets:

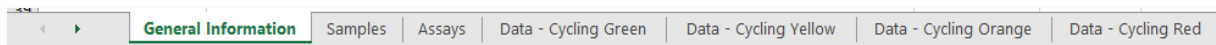


Fig. 99: Content of a run file, converted in an EXCEL worksheet

<b>General Information</b>	Contains information about the software version, file location, operator, start and finish times along with the run logs
<b>Samples</b>	The <b>Samples editor</b> is reproduced in Excel format.
<b>Assays</b>	Information about the chemistry type, target names, reporter dyes and channel used for each target, are shown for each assay run.
<b>Data Cycling</b>	The raw data for cycling is provided for each channel in a separate worksheet
<b>Analysis Results</b>	The analyzed results are presented in table format along with various graphs depending on the type of analyses selected in the run file. Each analysis is provided in a separate worksheet.
<b>Analysis - Data</b>	The processed data is provided.

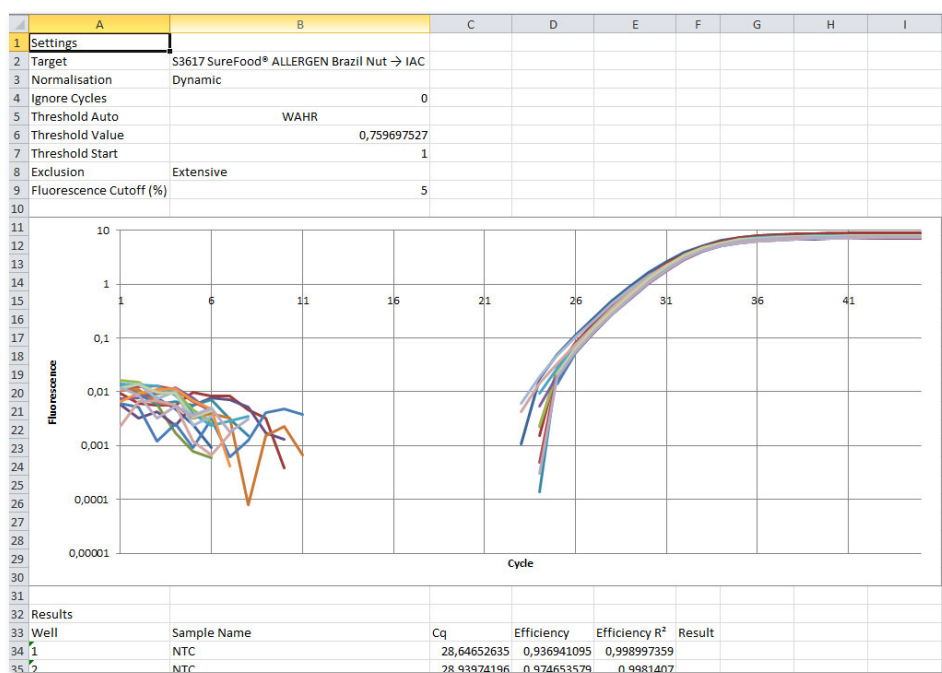


Fig. 100: Excel workbook Analysis Results

## Notes and Annex

### Technical data, ambient conditions

Dimensions	W: 150 mm, L: 150 mm, H: 130 mm (265 mm lid open)
Weight	2.1 kg
AC Input	100 - 240 VAC, 50/60 Hz 4.0 A
Temperature accuracy	± 0.25 °C
Temperature uniformity	± 0.05 °C
Ramp rates	Heating: 4 °C/s (fast mode) Cooling: 3 °C/s (fast mode)
Temperature input range	35 - 99 °C (min 40 °C when cycling)
Detectors	High sensitivity photodiode per channel
Excitation sources	High energy light emitting diode per channel
Channels	Green Ex. 465 nm Em. 510 nm filters
	Yellow Ex. 540 nm Em. 570 nm filters
	Orange Ex. 585 nm Em. 618 nm filters
	Red Ex. 635 nm Em. 675 nm filters
Acquisition time	1 s
Samples per instrument	48
Reaction volume range	5 - 30 µL
Environmental temperature	18 - 30 °C
Relative humidity	20 - 80 %
	Store in a cool, dry place in an upright orientation.

### Biological Safety information

Handle biological material with care and in accordance with the required safety regulations. Always wear safety glasses, gloves, and a lab coat. The user must take the necessary precautions to ensure that the surrounding workplace is safe and that the RIDA®CYCLER operators are suitably trained and not exposed to hazardous levels of infections agents.

## Decontamination of RIDA®CYCLER



### Decontamination

Cleaning and decontamination of the instrument is necessary as a safeguard when the instrument and any accessories are to be transferred to the manufacturer or certified maintenance body for repair, service or returns.

Surfaces of the RIDA®CYCLER, including the chamber and tube clamp, can be decontaminated using a solution of sodium hypochlorite (NaClO). A solution containing 1 gL<sup>-1</sup> available chlorine will be suitable for sanitation in a general lab environment; stronger solutions (5 gL<sup>-1</sup>) are recommended when dealing with high risk situations.

### Cleaning rotor wells:

The rotor wells of the RIDA®CYCLER can be cleaned using a solution of 70 -100 % isopropanol with an interdental brush (diameter of 5 mm and a minimum taper diameter of 1.9 mm)..

Cleaning the rotor wells can resolve issues such as fluorescent contamination from marker pens. A can of compressed air (preferably suited for camera optics) is recommended, too.

#### Procedure

- Open the lid and remove the magnetic tube clamp.
- Wet the interdental brush with the isopropanol.
- Insert the brush into a well and brush by rotating inside the well. Ensure a thorough brushing to remove any contaminant on the surface of the rotor well.
- After brushing allow the well to dry completely.
- Optionally, you can use a compressed air in a can to further clean out the well of any dust.
- If there is still some traces of contaminant left, repeat the procedure a further number of times.

In case of doubt, please contact your local distributor or R-Biopharm AG, Darmstadt directly.

## EC Declaration of conformity/CE Mark

The CE mark on the RIDA®CYCLER confirms that it meets all applicable requirements of the following European Directives:

Low Voltage Directive (LVD) 2006/95/EC

Electromagnetic Compatibility Directive (EMC) 2004/108/EC

And the relevant harmonized standards:

IEC 61010-1

IEC 61010-2-010

IEC 61010-2-081





ETSI EN 301 489-1 v1.9.2 (2011-09)

ETSI EN 301 489-17 v2.2.1 (2012-09)


EN 62311:2008

This declaration becomes void in case of any unauthorized modifications to the product.

## Type Plate Symbols

	<b>Regulatory Compliance Mark</b> This device is compliant with applicable ACMA technical standards for EMC.
	<b>FCC Declaration of Conformity</b> This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation. Contains FCCID: S7AIW03 Bluetooth® module, which can be found at the back of the instrument when the covers are removed
	<b>UL Listing</b> UL has tested representative samples of the product and determined that it meets UL's requirements for Laboratory Equipment.
	<b>CE Marking</b> The device is in compliance with the essential requirements and other relevant provisions of Low Voltage Directive 2006/95/EC.

## Disposal Information

	European Union (EU) directive for Waste Electrical and Electronic Equipment (WEEE).
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The disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.

Products sold in EU countries must be labeled with a crossed-out waste bin (or in individual cases, this must be marked on the packaging).

The WEEE Directive defines that customers and end users in EU countries (EU) must not dispose of electrical or electronic devices and electrical or electronic accessories in the household waste. Within the EU, please contact your local representative or your supplier's customer service, who can provide you with information about the disposal or collection of old devices.

## Annex 1: Temperature verification system (TVS)

The TVS can be used to ensure that the RIDA®CYCLER is operating to specification in regard to temperature control.



**Fig. 101:** Temperature verification system



**TVS sensor is fragile and connected via a very thin cable.**

Please handle with care to avoid breakage. Hold the device only by the sides.

### Verification Environment

For optimal results, it is best to perform the tests away from sources of airflow, for example fans and air vents. The ambient temperature may also have an effect on the measurement. We recommend to test under standard lab conditions (22 - 28°C).

### Hardware and Software Installation

- Connect the RIDA®CYCLER to be verified to your computer and switch it on.
- Take the TVS out of the protective case and connect it to your computer through USB.
- Open the RIDA®CYCLER software.  
Version must be compatible with used TVS device.


### Verification Process

- Once the RIDA®CYCLER and TVS are connected to your computer, scan for devices by clicking on the Communication icon.
- Select the instrument to test, and select **Temperature Verification** from the drop-down menu.
- Follow the prompts on the TVS Wizard.
- During these steps, keep the TVS placed beside your RIDA®CYCLER instrument.

**When prompted by the software, remove the sensor from the foam slot and place it in wells 1 – 4 with the tab facing inwards.**

Keep all other wells empty. Once the sensor is secured in with the tube clamp, close the lid and continue following the prompts on the screen. Verification may take up to 25 min.

**At the end, you will be given the option to save or print your verification report.**



**mic Temperature Verification Report**

Date Completed: 2 Dezember 2019  
 Instrument: M0001150  
 Verified By: TVS00020 (calibration valid until 13 September 2020)

Verification Results			
	mic Temp. (°C)	Actual Temp. (°C)	Measurement Error (Max±0,25°C)
Pt 60°C	60,01	60,02	-0,01
Pt 95°C	95,03	94,97	0,06

The verification data included in this document is traceable to a NATA certified reference.

This instrument has been verified to be working according to specification.

Name \_\_\_\_\_

Signature \_\_\_\_\_

**Fig. 102:** Temperature verification report

**Carefully remove the sensor from the instrument and place it back into the foam slot.**  
 Unplug the TVS from the computer and place it back into the protective case.

## Annex 2: Error messages and warnings

The following error messages and warnings are possible:

The selected assay is not compatible with the run.

The assay's profile and current run's profile do not match and cannot be automatically adjusted.

Warnings will be displayed if annotations have not been completed correctly

### Annex 3: Toshiba Bluetooth® work around

If you are using a Toshiba computer and you are not able to communicate with the RIDA®CYCLER instrument using Bluetooth® the following work around should rectify the issue.

1. Open the Device Manager.

In Windows® 8 point to the lower-right corner of the screen, move the mouse pointer up, and then click Search

Enter Device Manager in the search box, and tap or click Device Manager. You might be asked for an admin password or to confirm your choice.

In Windows® 7, click on the windows start button;

Select the control panel option;

Click on the "Hardware and Sound" heading;

Under the "Devices and Printers" heading, click on the "Device Manager".

2. Open the properties dialog for your Bluetooth® adapter.

Select the Bluetooth section header and a number of child items will appear.

In Windows® 8, select the Bluetooth VX Module (where X is a version number) as the Bluetooth® adapter.

In Windows® 7, select the "Bluetooth USB controller" device as the Bluetooth adapter.

If this device is not present, then you are probably not using the Toshiba Bluetooth drivers. You should not progress further with this trouble shooting process

3. Right click on the Bluetooth Adapter and select the "Update Driver Software" option.

4. Select the "Browse my computer for drive software" option.

5. Select the "Let me pick from a list of device drivers on my computer" option.

6. Select the "Generic Bluetooth Adapter" option.

7. Click on Next. Windows® will install the selected driver.

8. Windows® may ask to restart the computer. Before restarting, ensure all your data in all your applications is saved.

9. Restart the RIDA®CYCLER application. The Bluetooth devices should now be discovered

## Annex 4: CFR 21, Part 11

The Code of Federal Regulations Title 21 part 11 (21CFR11) of the U.S Food and Drug Administration, covers the regulations on electronic records and electronic signatures to ensure trustworthy, reliable and equivalent to paper records. We have implemented a number of features into our software that cover some of the requirements of 21CFR11 section 11.10 for closed systems. The procedures covered are listed and described below. The remaining procedures not covered by us, have a valid reason for each described below.

21CFR11 (section 11.10) states:

“Persons who use closed systems to create, modify, maintain, or transmit electronic records shall employ procedures and controls designed to ensure the authenticity, integrity, and, when appropriate, the confidentiality of electronic records, and to ensure that the signer cannot readily repudiate the signed record as not genuine. Such procedures and controls shall include the following”:

“(a) Validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records”.

The software contains run file signatures that ensure validity of the records. Any tampering of the run file through external software will break the signature, resulting in a notification by the software. The Data Integrity can be found in the Information page of the run file.



“(b) The ability to generate accurate and complete copies of records in both human readable and electronic form suitable for inspection, review, and copying by the agency”.

This control is met through the generation of pdf reports (see Reports for more information).

“(e) Use of secure, computer-generated, time-stamped audit trails to independently record the date and time of operator entries and actions that create, modify, or delete electronic records. Record changes shall not obscure previously recorded information. Such audit trail documentation shall be retained for a period at least as long as that required for the subject electronic records and shall be available for agency review and copying”.

The following will be logged and will appear in the Messages page of the application and the Event log section of the report or Excel® report when the run is saved or when a report is switched to:

- Changes to all parameters of analyses since the log update for analyses created before the last log update
- Changes from default parameter values for analyses created since the last log update
- Removal of analyses
- Changes in Analysis sample selection

There will be a single entry per analysis in the Message panel summarizing the changes. The time stamp of the entry is the time of the ‘Run Save’ not of the analysis parameter change.

The remaining procedures we have no control over, especially as some of them relate to the whole standard operating procedure for a test and/or quality management process, not just to the software. These include:

“(c) Protection of records to enable their accurate and ready retrieval throughout the records retention period”.

Provided by organization document management system (see note below).

“(d) Limiting system access to authorized individuals”.

Provided by organization document management system (see note below).

“(f) Use of operational system checks to enforce permitted sequencing of steps and events, as appropriate”.

This is an organization operational requirement.

“(g) Use of authority checks to ensure that only authorized individuals can use the system, electronically sign a record, access the operation or computer system input or output device, alter a record, or perform the operation at hand”.

Provided by organization document management system (see note below).

“(h) Use of device (e.g. terminal) checks to determine, as appropriate, the validity of the source of data input or operational instruction”.

This is an organization operational requirement.

“(i) Determination that persons who develop, maintain, or use electronic record/electronic signature systems have the education, training, and experience to perform their assigned tasks”.

This is an organization operational requirement.

“(j) The establishment of, and adherence to, written policies that hold individuals accountable and responsible for actions initiated under their electronic signatures, in order to deter record and signature falsification”.

This is an organization operational requirement.

“(k) Use of appropriate controls over systems documentation including:

- (1) Adequate controls over the distribution of, access to, and use of documentation for system operation and maintenance.
- (2) Revision and change control procedures to maintain an audit trail that documents time-sequenced development and modification of systems documentation”.

This is an organization operational requirement.

File management is up to the end user. They must configure appropriate permissions and file storage such that it meets these requirements. Windows allows you to have separate permissions to create files or write data and delete. However, it does not distinguish between writing a file and overwriting a file so there is nothing to prevent you from saving one file over the top of another.

It would be our recommendation to use a proper enterprise quality management software system like MasterControl™ (MasterControl Inc., Utah, USA) to meet all the 21CFR11 requirements.

**Annex 5: Dye color chart**

Dye	Excitation	Emission	Channel	Application
BEBO	468	492		Intercalating
LC Green®	455	495		HRM dye
SYTO® 9	483	503		HRM dye
FAM™ (optimal)	494	515		Conjugated label
SYBR® Green I	494	521		Intercalating
RiboGreen®	500	520		RNA label
PicoGreen®	502	523		ds DNA label
Eva Green®	503	527		HRM dye
TET™	521	536	suboptimal	Conjugated label
CAL Fluor® Gold 540	522	541	suboptimal	Conjugated label
JOE™	520	548	suboptimal	Conjugated label
VIC®	538	554		Conjugated label
HEX™	535	555		Conjugated label
CAL Fluor Orange 560 (optimal)	540	561		Conjugated label
Quasar® 570	548	566		Conjugated label
Cy™3	550	570		Conjugated label
NED™	546	575		Conjugated label
TAMRA™	555	576		Conjugated label
CAL Fluor® Red 590	565	588	x	Conjugated label
ROX™	573	602		Conjugated label
Texas Red®	583	603		Conjugated label
CAL Fluor® Red 610 (optimal)	590	610		Conjugated label
LC® Red 640	620	635	suboptimal	Conjugated label
Quasar® 670 (optimal)	647	667		Conjugated label
Cy™ 5	651	674		Conjugated label
Cy™5.5	675	694		Conjugated label
Quasar® 705	690	705	x	Conjugated label

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**Abbreviations**

CI	Confidence interval
Cq	Quantification cycle
CV	Coefficient of variation
EC	Extraction control
IAC	Internal amplification control
LoD	Limit of detection
NTC	No template control
PCR	Polymerase chain reaction
PTC	Positive template control
qPCR	Real time quantitative PCR
RIN	RNA integrity number
RT-qPCR	Reverse transcription real time quantitative PCR
SD	Standard deviation
W-o-L	Window of linearity

## Glossary

Analytical Accuracy	the difference between the experimentally measured and actual concentrations.
Analytical Repeatability:	precision of the assay within the same samples repeatedly measured in the same assay. Also, referred to intra-assay variance it is expressed as the SD for the Cq variance or CV of the copy number/ concentration variance.
Analytical Reproducibility	the variation in results between runs or different laboratories. It is also referred to as inter-assay variance and is expressed as the SD or CV of copy number or concentration. As Cq's typically vary between runs the reporting of inter run variation is not appropriate.
Analytical sensitivity	the minimum number of copies in a sample that can be measured accurately with an assay.
Analytical specificity	assay detecting the specific target sequence rather than another, nonspecific, target. Use of NTC's helps determine analytical specificity.
Hydrolysis probes	are short oligonucleotides with a fluorescence reporter dye at the 5' end and a quencher molecule at the 3' end. When the probe is intact, the close proximity of the reporter dye to the quencher results in little fluorescence being detected. During extension, the polymerase will cleave the probe through exonuclease activity separating the reporter dye from the quencher.
Limit of Detection (LoD)	the LoD is the minimum concentration that can be detected with reasonable certainty (typically 95 % probability).
Linear dynamic range	is the highest to the lowest quantifiable copy number determined by means of a standard curve
Magnetic induction	when a conductor such as a metal is exposed to a magnetic field it produces a circular electric current, also known as an eddy current, in the conductor. Due to the resistance of the conductor it becomes hot. In the RIDA®CYCLER instrument the rotor is the conductor that becomes hot under as it is exposed to a magnetic field.



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